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IODINE BALANCE IN EXOPHTHALMIC GOITER

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Hyperthyroidism augments the metabolism of several of the body components. It has long been shown that hyperthyroidism accentuates the basal rate of combustion of carbohydrate, fat and at times protein. Its effect on consumption of oxygen and production of carbon dioxide is well known. Its accentuation of the metabolism of calcium,[1] phosphorus [1a] and nitrogen [2] has also been shown. But of primary significance in the consideration of disturbed thyroid function is the effect of hyperthyroidism on the metabolism of iodine. Iodine is an essential component of the thyroid hormone. Thyroid function and iodine metabolism are thus intimately related. A disturbance of the one may lead to a disturbance of the other.

Extensive studies of the iodine of the blood [3] and urine [4] both of normal persons and of patients with exophthalmic goiter have been reported. These revealed definite hyperiodemia and hyperioduria in patients with exophthalmic goiter. Data concerning the iodine balance of 2 patients with untreated exophthalmic goiter have been presented.[5] A review of the pertinent literature was included.[6] These data gave

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1. (*a*) Aub, J. C.; Bauer, W.; Heath, C., and Ropes, M.: J. Clin. Investigation **7**:97, 1929. (*b*) Puppel, I. D., and Curtis, G. M.: Arch. Int. Med. **58**: 957, 1936.

2. (*a*) Müller, F.: Deutsches Arch. f. klin. Med. **51**:335, 1893. (*b*) Boothby, W. M.: M. Clin. North America **5**:425, 1921; Minnesota Med. **7**:91, 1924. (*c*) Boothby, W. M., and Sandiford, I.: J. A. M. A. **81**:795, 1923.

3. Curtis, G. M.; Davis, C. B., and Phillips, F. J.: J. A. M. A. **101**: 901, 1933. Curtis, G. M.; Cole, V. V., and Phillips, F. J.: West. J. Surg. **42**:435, 1934. Davis, C. B.; Curtis, G. M., and Cole, V. V.: J. Lab. & Clin. Med. **19**:818, 1934.

4. (*a*) Curtis, G. M.; Puppel, I. D.; Cole, V. V., and Matthews, N. L.: J. Lab. & Clin. Med. **22**:1014, 1937. (*b*) Curtis, G. M., and Puppel, I. D.: Arch. Int. Med. **60**:498, 1937; (*c*) West. J. Surg. **45**:417, 1937.

5. Cole, V. V., and Curtis, G. M.: J. Nutrition **10**:493, 1935.

6. Curtis and others.[3] Curtis and others.[4] Cole and Curtis.[5]

incomplete evidence of the effect of hyperthyroidism on the total iodine metabolism. Consequently, we have investigated the iodine balance of 4 normal persons under various conditions over a total period of eighty-seven days and that of 10 patients with various thyroid diseases over a total period of two hundred and eighty-five days. Data which we are about to present demonstrate that in patients with exophthalmic goiter there is increased mobilization of iodine, with elevation of blood iodine and increase in excretion of iodine through one or all excretory channels. This results in profound disturbance of the iodine balance.

METHODS

The experimental and laboratory methods used, except for certain modifications, have been set forth in a previous publication.[1b] A constant regimen of experimental and hospital management, which was maintained throughout the period of study, was begun from five to six days prior to investigation. This was to establish a preliminary period of exogenous control of iodine metabolism. Even with a constant food regimen the intake of iodine in food varied moderately. This, however, was relatively constant in comparison with the other variables of the iodine balance, as will soon be noted. The intake of iodine was maintained low throughout the investigation. Twenty-four hour collections of urine were taken and measured, and a specimen of a pooled three day collection was analyzed.

The collection of sweat iodine was carried out as follows: Prior to the start of the first period of investigation, the body was washed with distilled water and liniment of soft soap U. S. P. and then rinsed. This water was discarded. Underwear and stockings which had been washed thoroughly with iodine-free distilled water were worn by the patient. Face washings were made daily with 500 cc. of distilled water and 5 cc. of liniment of soft soap U. S. P., the iodine content of which had been previously determined. At the end of each three day period, the underwear and stockings were washed and then soaked for twelve hours in 7,500 cc. distilled water and 15 cc. of liniment of soft soap. The patient was bathed with 500 cc. of distilled water and 5 cc. of liniment of soft soap at the termination of each three day period. The body was then rinsed with 500 cc. of distilled water. The washings and bath water for each period were pooled for analysis.

The accuracy of this method of collecting sweat iodine was tested by washing and bathing a patient as in preparation for the usual study of balance. Forty-two micrograms of iodine as aqueous iodate was then applied to various parts of the patient's skin. The patient was then washed and bathed as in the procedure just described for collecting sweat iodine. Ninety-eight per cent of this added iodine was recovered.

The blood, urine, feces and sweat were analyzed for iodine by Matthews'[7] modification of the Leipert[8] procedure. Three separate daily samples of the constant diet taken by each patient were analyzed for iodine by a combination of the Von Kolnitz-Remington[9] method and the Matthews[7] method. Otherwise the methods of preparation of the diet, collection of the excreta and chemical

7. Matthews, N. L.: Master's Thesis, Ohio State University, August 1937. Matthews, N. L.; Curtis, G. M., and Brode, W. R.: Indust. & Engin. Chem. (Anal. Ed.), Oct. 15, 1938.

8. Leipert, T.: Biochem. Ztschr. **261**:436, 1933; **270**:448, 1934.

9. Von Kolnitz, H., and Remington, R. E.: Indust. & Engin. Chem. (Anal. Ed.) **5**:38, 1933.

analysis of the specimens have been described.[1b] For determining the iodine in the air we used Scheffer's method.[10] However, this has not proved entirely satisfactory in our hands. We were unable to recover within 10 per cent a known amount of gaseous iodine liberated in the Douglas bag and KOH = H_2SO_3 train system. Consequently, we do not include our findings on the iodine in the air in this report. These will be reported later.[11]

NORMAL IODINE BALANCE

We have studied 3 normal persons as controls (tables 1 and 2 and chart 1) under various conditions over a period of thirty-nine days. These persons were maintained during twenty-four days on a low intake of iodine, averaging 87 micrograms per three day period. They remained in continuous negative iodine balance throughout the thirty-nine days. One (R. B.) showed an increase in negative iodine balance during a period of starvation lasting six days.

Protocols may be briefly presented as follows:

R. B., a 19 year old white man, single, was readmitted to the research surgery service Nov. 6, 1936, for repair of a bilateral inguinal hernia. He had received no iodine or thyroid medication in any form. Physical examination showed normal conditions throughout except for relaxed external inguinal rings.

The Wassermann and Kahn reactions of the blood were negative. The blood showed monocytosis. The urine was normal. The phenolsulfonphthalein test for renal function was normal. The blood urea nitrogen was 11.5 mg. per hundred cubic centimeters. The basal metabolic rate on November 7 was minus 11, with the basal pulse rate 71, the temperature 97.2 F., the respiratory rate 15, the blood pressure 112 systolic and 88 diastolic and the weight 149 pounds (67.6 Kg.).

Studies of iodine and of calcium balance were made from November 17 to December 17. Food starvation was maintained from November 26 to December 2, with a subsequent recovery period of six days. Repair of the right inguinal hernia was accomplished on December 8. The postoperative course was uneventful. The patient was discharged December 22.

Comment.—This young man maintained on a low intake of iodine (98 micrograms per three day period) remained in continuous negative iodine balance, averaging 95 micrograms per three day period, throughout the two periods of study (table 1). This was particularly due to excretion of iodine in the urine. There was less loss through feces and sweat. The blood iodine was low normal (table 1).

Food was then completely withdrawn for six days, during which only iodine-free distilled water was allowed (table 1). Excretion of iodine in the urine and sweat continued. Doubtless excretion into the gastrointestinal tract continued, as is evidenced in the feces of the period immediately subsequent to termination of the starvation. This may have resulted from the washing-out effect of the first food subsequently passing along the gastrointestinal tract; in any case an even greater negative balance ensued (table 1). The iodine excreted, how-

10. Scheffer, L.: Klin. Wchnschr. **13**:1570, 1934.

11. Puppel, I. D.; Davison, R. A., and Curtis, G. M.: Unpublished data.

TABLE 1.— *Data for R. B., a Normal White Man Aged 19*

Period	Date When Started, 1936	Weight, Kg.	Iodine: Output: Urine, Mcg.*	Iodine: Output: Feces, Mcg.	Iodine: Output: Sweat, Mcg.	Iodine: Output: Total, Mcg.	Iodine: Intake, Mcg.	Iodine: Balance, Mcg.	Date, 1936	Blood Iodine Mcg. per 100 Cc.	B.M.R., Percentage	Comment
1	11/17	68	133	23	39	195	101	—94	11/17	3.2	—10	
2	11/20	68	110	44	36	190	95	—95	11/20	1.4	—14	
3	11/26	68	94	0	40	134	0	—134	11/26	2.8	—21	Food starvation from November 26 to December 2
4	11/29	64	58	0	55	113	0	—113	11/28	1.4	—17	
									11/30	0.9	—18	
5	12/ 2	63	117	315	41	473	98	—375	12/ 1	...	....	
									12/ 2	...	—27	
6	12/ 5	66	109	16	36	161	98	—63	12/ 5	3.8	—37	
7	12/ 8	65	2,386	41	107	2,534	81	—2,453	12/ 8	2.7	—28	Right herniorrhaphy December 8
8	12/11	65	978	38	53	1,069	81	—988	12/11	3.5	—11	
									12/14	2.8	— 5	
9	12/14	65	183	35	65	286	98	—188	12/17	4.0	—25	

* A microgram (mcg.) equals 0.001 mg.

TABLE 2.—*Data for J. R., a Normal White Man Aged 56*

Period	Date When Started, 1936	Weight, Kg.	Iodine: Output: Urine, Mcg.*	Iodine: Output: Feces, Mcg.	Iodine: Output: Sweat, Mcg.	Iodine: Output: Total, Mcg.	Iodine: Intake, Mcg	Iodine: Balance, Mcg.	Date, 1936	Blood Iodine Mcg. per 100 Cc.	B.M.R., Percentage	Comment
			Diet Constant, Totaling 2,000 Calories and 80 Gm. of Protein									
1	10/15	59	153	41	28	222	77	—145	10/15	4.6	—10	Rest in bed
									10/17	4.0	—14	
2	10/18	59	153	30	21	204	74	—130	10/19	3.8	—13	
									10/21	5.3	—11	
3	10/21	59	157	32	26	215	61	—154	10/23	4.5	—16	
			Diet Supplemented with Iodized Milk									
1	10/24	59	1,010	102	23	1,135	1,608	+473	10/27	6.9	—13	Iodized milk given, 750 cc. daily
2	10/27	59	1,340	97	29	1,466	1,698	+232	10/29	3.8	—11	
			Diet Supplemented with Iodine as Potassium Iodide									
1	10/30	60	5,960	51	47	6,078	9,078	+3,000	10/30	6.4	—13	Iodine administered, 3 mg. daily, from October 30 to November 5
2	11/ 2	60	7,200	66	70	7,336	9,078	+1,742	11/ 2	9.3	— 7	
3	11/ 5	60	14,600	111	112	14,823	18,078	+3,255	11/ 5	17.3	— 8	Iodine administered, 6 mg. daily, from November 5 to 11
4	11/ 8	59	14,300	181	123	14,604	18,078	+3,474	11/ 8	15.0	— 7	
									11/11	16.6	....	Iodine administered, 12 mg. daily, from November 11 to 14
5	11/11	59	26,600	173	193	26,966	36,078	+9,112	11/14	29.6	....	

* A microgram (mcg.) equals 0.001 mg.

ever, reached a minimum during the last of the period of starvation and might have decreased still further if the fast had been prolonged.

After the patient's recovery from starvation a right herniorrhaphy was done. Immediately after operation there ensued a great increase in the excretion of iodine (table 1), particularly in the urine. A great increase in negative iodine balance was consequently noted. This had returned toward normal as early as the sixth day postoperatively (table 1).

J. R., a white man of 56 years, unmarried, was readmitted to the University Hospital Sept. 4, 1936, for surgical management of right recurrent inguinal hernia. Orchidectomy on the right had been done previously. An iodine tolerance test was made June 22 with 10 mg. of iodine as potassium iodide. The patient received milk with an increased iodine content from June 26 to July 23. During the study of iodine balance he had a mild infection of the upper respiratory tract. Examination revealed right recurrent inguinal hernia and absence of the right testicle.

The Wassermann and Kahn reactions of the blood were negative. The blood and urine were normal. The blood urea nitrogen on October 6 was 8 mg. per hundred cubic centimeters. Renal function was normal, as evidenced by the phenolsulfonphthalein test. The basic metabolic rate October 15 was —10 per cent, with the basal pulse rate 71, the temperature 97 F., the respiratory rate 16, the blood pressure 142 systolic and 84 diastolic and the body weight 130 pounds (59 Kg.).

Studies of calcium and iodine balance were made from October 15 to November 17. Milk with an increased iodine content [12] was administered from October 24 to 30. Potassium iodide solution was administered in increasing doses from October 30 to November 17. The hernia was repaired December 21. The postoperative convalescence was uneventful. The patient was discharged Jan. 19, 1937.

Comment.—This normal man maintained on a low intake of iodine (70 micrograms per three day period) for nine days showed a continuous negative iodine balance. This averaged 143 micrograms per three day period (chart 1). The blood iodine was normal (chart 1).

I. E., a white woman of 26 years, married, was transferred to the research ward Feb. 7, 1937. She was eight months pregnant. Her first pregnancy had ended at six months, in August 1935, and her second pregnancy had resulted in a miscarriage at four months, in March 1936. The cause of these two spontaneous abortions was not clear. The general health of the patient had been good until the present pregnancy. During the first three months there had been moderate nausea and vomiting. During mid-January a severe infection of the upper respiratory tract developed, with cough and expectoration. This had subsided completely at the time of our investigation. In September 1936 she began to take desiccated thyroid and corpus luteum and continued to do this up to Jan. 27, 1937. Physical examination yielded negative results throughout except for the characteristic findings of an eight months' pregnancy.

The Wassermann and Kahn reactions of the blood were negative. The urine was normal. The blood February 15 showed microcytic, hypochromic anemia, with

12. This was obtained from a dairy herd which had been maintained on a feed with supplemental iodine content.

the erythrocytes at 3,800,000 per cubic millimeter and hemoglobin at 7.6 Gm. per hundred centimeters (Newcomer). Otherwise the blood findings were normal. The phenolsulfonphthalein test for renal function was normal. The basal metabolic rate February 10 was + 7 per cent, with the basal pulse rate 104, the respiratory rate 16, the temperature 97.6 F., the blood pressure 100 systolic and 58 diastolic and the body weight 141 pounds (94 Kg.).

Studies of iodine and calcium balance were made from February 13 to 22. Treatment of the anemia with iron was begun March 1. April 6 the erythrocytes were 4,780,000 per cubic millimeter and the hemoglobin 17 Gm. per hundred cubic centimeters (Newcomer). The patient was delivered of a full term normal girl April 3. Both were discharged April 13.

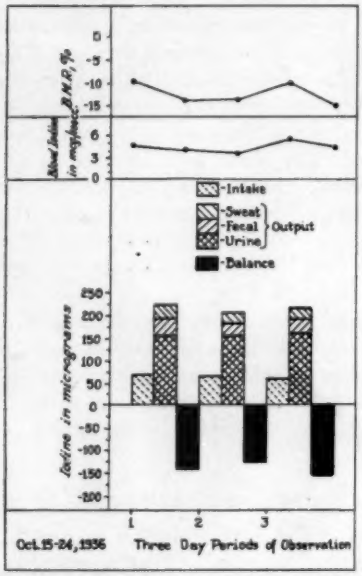

Chart 1.—The iodine balance of a normal man, J. R., aged 56, while he was on a constant regimen. Note the continuous negative balance on a low intake.

Comment.—During the eighth month of gestation this woman showed a continuous normal negative iodine balance averaging 129 micrograms per three day period over nine days (table 3). The blood iodine, however, was increased (table 3).[13]

IODINE BALANCE IN EXOPHTHALMIC GOITER

The iodine balance of 5 patients with exophthalmic goiter was investigated pretherapeutically over a period of sixty-three days (charts 2, 4, 5 and 6 and table 5). Three patients with exophthalmic goiter

13. Maurer, E., and Diez, S.: München. med. Wchnschr. **73**:17, 1926. Scheringer, W.: Arch. f. Gynäk. **143**:319, 1930.

TABLE 3.—*Data for I. E., a Woman Aged 26, Eight Months Pregnant, on a Diet Totaling 2,170 Calories and 49 Gm. of Protein*

			Iodine									
			Output									
Period	Date When Started, 1937	Weight, Kg.	Urine, Mcg.*	Feces, Mcg.	Sweat, Mcg.	Total, Mcg.	Intake, Mcg.	Balance, Mcg.	Date, 1937	Blood Iodine Mcg. per 100 Cc.	B.M.R., Percentage	Comment
1	2/13	64.5	163	28	25	216	104	—110	2/12	7.4	+7	Patient given general hospital management
									2/15	5.9	+8	
2	2/16	64.1	194	18	31	243	105	—138	2/17	10.9	+5	
3	2/19	64.5	166	32	18	216	77	—139	2/22	5.6	+3	

* A microgram (mcg.) equals 0.001 mg.

(charts 2 and 4 and table 5) who were maintained on a low intake of iodine, averaging 87 micrograms per three day period, which was similar to that of our normal controls, revealed a negative iodine balance greater than the normal. This resulted from increased mobilization and loss of iodine, particularly through the renal and gastrointestinal tracts.

Two other patients (charts 5 and 6) with exophthalmic goiter were maintained on sufficient iodine to keep a normal person in positive iodine balance. However, these patients also showed negative iodine balance.

Protocols may be briefly presented as follows:

F. W., a 23 year old white man, unmarried, was hospitalized July 21, 1936, for management of exophthalmic goiter. The clinical picture was typical and included exophthalmos, goiter, tachycardia and tremor. Prominence of the eyes and beginning goiter were noted in the preceding January, when progressive symptoms caused him to consult a physician, who recognized exophthalmic goiter. Symptoms had been noted for approximately two years, during which time he had lost 47 pounds (21.3 Kg.) in weight. There were no associated symptoms due to pressure.

Physical examination revealed a nervous, restless young man who was manifestly poorly nourished. Exophthalmometric readings were, right, 23 and, left, 22 mm. The thyroid was diffusely enlarged bilaterally and presented a pronounced bruit. The trachea was deviated to the left. Tremor was obvious.

The Wassermann and Kahn reactions of the blood were negative. The blood September 19 was normal except for a lymphocytosis. The hemoglobin content was normal. The urine was normal on four occasions. The phenolsulfonphthalein test showed normal renal function. The blood urea nitrogen on October 6 was 9.5 mg. per hundred cubic centimeters. The basal metabolic rate on October 7 was + 84 per cent, with the basal pulse rate 115, the respiratory rate 14, the temperature 98 F., the blood pressure 142 systolic and 60 diastolic and the body weight 108 pounds (49 Kg.).

Studies of iodine and calcium balance were made from October 15 to December 17. Milk with an increased iodine content and iodine as potassium iodide were administered, the latter in gradually increasing doses (table 4), and their effect on the clinical and laboratory findings was noted. Previous to the commencement of these studies the patient had received an extract of adrenal cortex in increasing doses from August 15 to September 1. Subsequently he had been given milk with an increased iodine content, 1 liter daily, from September 15 to 17 and 1,500 cc. daily from September 17 to 30.

The treatment consisted of rest in bed, iodinization and eventual thyroidectomy in two stages. A subtotal right lobectomy was done November 30. Postoperatively, the administration of 48 mg. of iodine as potassium iodide by mouth daily was continued. Convalescence was uneventful except for an exacerbation of an old chronic left otitis media with a purulent discharge. This subsequently cleared. A left subtotal lobectomy was done December 8. Convalescence was uneventful. The postoperative management by means of iodine was identical with that of the first stage. The basal metabolic rate December 21 was + 8 per cent. The patient was discharged December 23, the fifteenth day after the second operative stage.

TABLE 4.—*Data for F. W., a Man Aged 23 Years with Exophthalmic Goiter*

Period	Date When Started, 1936	Weight, Kg.	Iodine Output: Urine, Mcg.*	Iodine Output: Feces, Mcg.	Iodine Output: Sweat, Mcg.	Iodine Output: Total, Mcg.	Iodine Intake, Mcg.	Iodine Balance, Mcg.	Date, 1936	Blood Iodine Mcg. per 100 Cc.	B.M.R., Percentage	Comment
						Diet Constant, Totaling 3,100 Calories and 85 Gm. of Protein						
1	10/15	48	323	354	57	734	83	—651	10/15	14.6	+75	Rest in bed
									10/17	12.7	+88	
2	10/18	48	305	255	61	621	67	—554	10/19	12.2	+75	
									10/21	12.8	+94	
3	10/21	49	290	257	41	588	72	—516	10/23	11.6	+74	
						Diet Supplemented with Iodized Milk						
1	10/24	49	333	354	24	711	1,627	+916	10/25	11.7	+74	Iodized milk given, 750 cc. daily
									10/27	10.7	+76	
2	10/27	49	390	185	50	625	1,708	+1,083	10/29	9.9	+80	
						Diet Supplemented with Iodine as Potassium Iodide						
1	10/30	49	3,040	385	38	3,463	9,071	+5,608	10/31	12.8	+73	Iodine given, 3 mg. daily, from October 30 to November 5
2	11/ 2	49	1,913	285	34	2,232	9,071	+6,839	11/ 2	10.4	+72	
3	11/ 5	49	8,420	249	152	8,821	18,071	+9,250	11/ 5	12.7	+71	Iodine given, 6 mg. daily, from November 5 to 11
4	11/ 8	48	7,370	244	153	7,767	18,071	+10,304	11/ 8	11.3	+71	
5	11/11	49	21,400	310	302	22,012	36,071	+14,059	11/11	11.8	+68	Iodine given, 12 mg. daily, from November 11 to 17
6	11/14	49	21,000	340	314	21,654	36,071	+14,417	11/14	12.4	+72	
7	11/17	49	47,000	560	840	48,400	72,071	+23,671	11/17	13.2	+64	Iodine given, 24 mg. daily, from November 17 to 23
8	11/20	49	53,100	870	990	54,960	72,071	+17,111	11/20	33.1	+42	
9	11/23	48	125,000	1,180	1,780	127,960	144,071	+16,111	11/23	31.0	+62	Iodine given, 48 mg. daily, from November 23 to 26
10	11/26	48	98,100	3,563	2,480	104,133	144,071	+39,938	11/26	39.0	+48	
						Iodine Therapy Followed by Surgical Treatment for Exophthalmic Goiter						
1	11/29	48	121,980	1,205	2,880	126,065	144,071	+18,006	11/29	56.0	+67	Iodine given, 48 mg. daily, from November 26 to December 17
2	12/ 2	..	122,500	480	2,220	125,200	144,071	+18,871	12/ 2	24.0	....	Subtotal right lobectomy on November 30
3	12/ 5	47	118,700	394	2,690	121,784	144,071	+22,287	12/ 5	35.0	+40	
4	12/ 8	46	120,300	270	4,040	124,670	144,071	+19,401	12/ 8	33.0	+36	Subtotal left lobectomy on December 8
5	12/11	46	135,000	298	3,340	138,633	144,071	+5,438	12/11	61.0	+42	
									12/14	39.0	+18	
6	12/14	46	134,000	182	2,450	136,632	144,071	+7,439	12/17	37.0	+21	

* A microgram (mcg.) equals 0.001 mg.

One hundred and fifty grams of characteristic diffuse hyperplastic goiter was removed. Microscopic examination showed extensive, generalized iodine-induced colloid involution. The removed tissue contained approximately 100 mg. of iodine, which is about ten times the normal.

Comment.—This young man presented severe exophthalmic goiter, verging on a thyroid crisis. The basal metabolic rate ranged from + 75 to + 94 per cent during our early investigation. On a low intake of iodine, 74 micrograms per three day period, the negative iodine balance remained continuously increased, from four to five times greater than normal, over a total period of nine days and averaged 574 micrograms per three day period (chart 2).

E. C., a white schoolgirl of 24, entered the University Hospital March 16, 1937, for management of exophthalmic goiter. She presented the characteristic clinical and laboratory features of this disease. She had been "nervous" throughout life. However, about six weeks previous to admission she noted that her "nervousness" was increasing. Easy fatigability, palpitation, easy excitability, headaches and loss of appetite, with occasional nausea and vomiting, ensued. There followed a loss of weight of 15 pounds (6.8 Kg.) and tremor of the hands. She noted slight exophthalmos. She had not noted enlargement of her neck. She had not received iodine or thyroid medication. No symptoms caused by pressure were evident.

Physical examination revealed a well developed but poorly nourished and unusually apprehensive young woman, weighing 88 pounds (39.9 Kg.). Exophthalmometric readings were, right, 18 and, left, 16 mm. Goiter was not noticeable on inspection; however, to palpation the thyroid gland presented slight diffuse bilateral enlargement. The trachea was in a normal position. The pulse rate was 119. The tremor was unusual.

The Wassermann and Kahn reactions of the blood were negative. The blood was normal save for the hemoglobin, which was 10.9 Gm. per hundred cubic centimeters (Newcomer). The urine was normal. The blood urea nitrogen was 15 mg. per hundred cubic centimeters. The phenolsulfonphthalein test showed the renal function as normal. The basal metabolic rate March 18 was + 44 per cent, with the basal pulse rate 107, the respiratory rate 15, the temperature 97.2 F., the blood pressure 116 systolic and 54 diastolic and the body weight 89 pounds (40.4 Kg.).

On medical management alone, including rest in bed, a diet high in calories and calcium therapy, the patient showed symptomatic improvement and a decreasing basal metabolic rate, to + 12 per cent on April 23, with the basal pulse rate 83, the respiratory rate 22, the temperature 97.2 F., the blood pressure 102 systolic and 50 diastolic and the weight 86 pounds (39 Kg.). Menstruation occurred from April 17 to 19.

April 24 iodine therapy was instituted, 10 mg. of iodine as potassium iodide being given daily for nine days. The basal metabolic rate was further decreased and established at + 0. per cent. Symptomatic improvement continued. A vacation in the medication with iodine was then maintained from May 2 to June 11. During this interval the symptoms recurred, and the basal metabolic rate gradually increased.

TABLE 5.—*Data for E. C., a Woman Aged 24, with Incipient Exophthalmic Goiter, Who Was on a Diet Totaling 2,420 Calories and 49 Gm. of Protein*

			Iodine									
			Output									
Period	Date When Started, 1937	Weight, Kg.	Urine, Mcg.*	Feces, Mcg.	Sweat, Mcg.	Total, Mcg.	Intake, Mcg.	Balance, Mcg.	Date, 1937	Blood Iodine Mcg. per 100 Cc.	B.M.R., Percentage	Comment
1	4/ 6	40	106	102	53	261	87	—174	3/23	...	+45	Patient given general hospital management, including
									4/ 5	6.4	+26	rest in bed and a diet high in calories
2	4/ 9	40	99	89	32	220	87	—133	4/ 8	6.9	+20	
3	4/12	40	104	113	39	256	84	—172	4/11	...	+33	
4	4/15	40	94	133	37	264	87	—177	4/14	5.8	+24	Calcium lactate given, 50 Gm. daily, from April 15 to 18 Menstruation from April 17 to 19

* A microgram (mcg.) equals 0.001 mg.

Supplemental roentgen therapy was begun on May 18, at which time the patient received 300 roentgens to each lobe. This was repeated May 21. May 27, 300 roentgens was given to the left lobe and May 28, 300 roentgens to the right lobe; June 2, 300 roentgens were given again to the left lobe and June 4, 300 roentgens to the right lobe. The patient showed no immediate clinical or laboratory improvement. The basal metabolic rate was established at about + 30 per cent.

June 11 iodine therapy was reinstituted, with 10 mg. of iodine as potassium iodide being administered daily for fifteen days. Immediate improvement in the

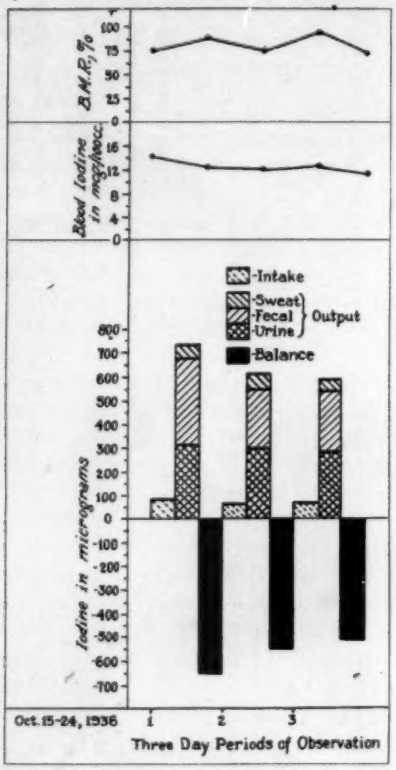

Chart 2.—The iodine balance of F. W., a man aged 23, who had severe exophthalmic goiter and who was on a constant regimen. Note that on a low intake similar to that of the normal person (J. R., chart 1) there was greatly increased excretion of iodine. This resulted in a negative iodine balance approximately four times the normal.

clinical status ensued. The basal metabolic rate decreased to + 7 per cent on June 25, with the basal pulse rate 81, the respiratory rate 19, the temperature 97.2 F., the blood pressure 112 systolic and 62 diastolic and the body weight 86 pounds (39 Kg.). The investigation of the calcium and iodine balance was made from April 6 to June 26.

A varied regimen of nonoperative treatment was thus followed, including rest in bed, a diet high in calories, iodinization and supplementary roentgen therapy. The patient was discharged June 27, 1937.

Comment.—This young woman showed a remission of the clinical symptoms on hospital management alone. The basal metabolic rate gradually decreased to within normal limits—from + 9 to + 14 per

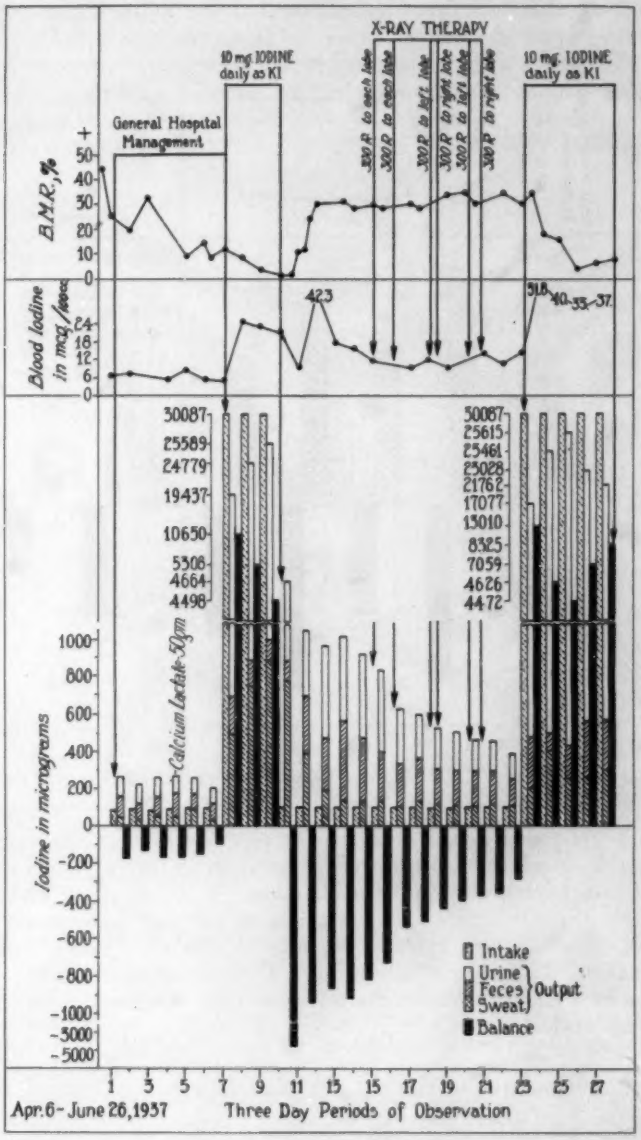

Chart 3.—The behavior of the iodine balance of E. C., a woman aged 24, with exophthalmic goiter, during general hospital management, iodinization, "iodine vacation," roentgen therapy and reiodinization.

cent. On a low intake of iodine, averaging 86 micrograms per three day period, there was at first a moderately increased negative iodine balance, averaging 164 micrograms per three day period (table 5). There occurred then a gradual decrease in excretion of iodine through all excretory channels investigated, so that the iodine balance returned

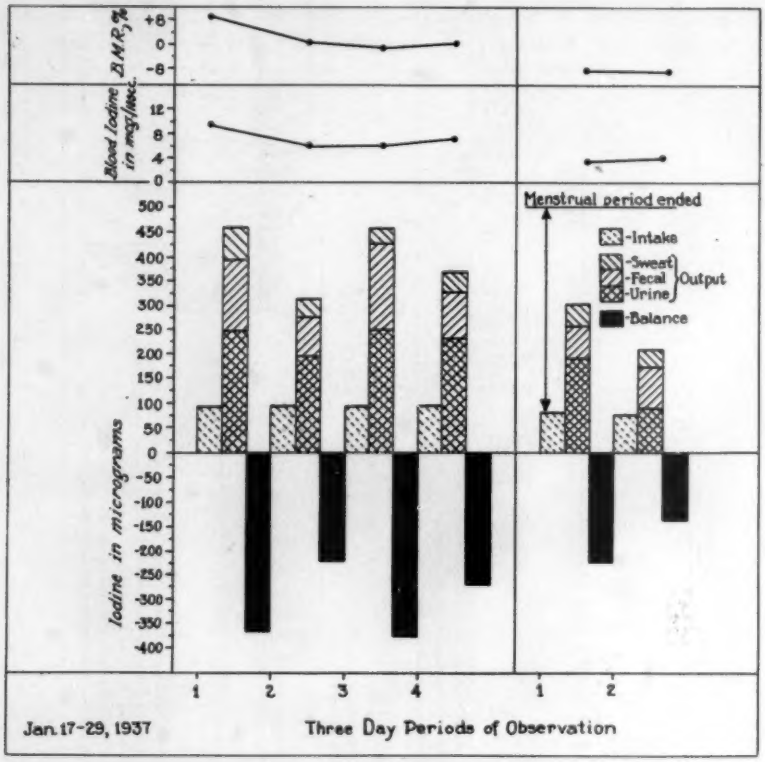

Chart 4.—A comparison of the iodine balance in a patient with exophthalmic goiter (M. W., at left) after inadequate operation on the thyroid (single lobectomy) with that in a patient with exophthalmic goiter (T. F., at right) after adequate operation on the thyroid. M. W. was a girl of 15 years with recurrent hyperthyroidism. She had been operated on seven months prior to the study of her iodine balance: T. F. was a woman aged 27 who had been relieved of hyperthyroidism by a bilateral subtotal thyroidectomy done two years and seven months prior to this study.

to within normal limits during the sixth three day period of study (chart 3).

M. W., a white schoolgirl of 15, was readmitted to the research surgery service Jan. 4, 1937, for investigation of the iodine and calcium balance subsequent to a right subtotal thyroid lobectomy May 18, 1936.

She had improved since her discharge July 1, 1936; however, there remained persistent hyperthyroidism. Nervousness, easy perspiration, palpitation, dyspnea on exertion and increased appetite were still noted. The basal metabolic rate remained elevated. The entire clinical picture, however, was not so severe as preoperatively. She had been given no iodine medication since her discharge on July 1. During the present hospital investigation a decrease in the basal metabolic rate from + 33 per cent on January 3 to —3 per cent on January 31 occurred together with partial relief from the hyperthyroidism.

Examination revealed an easily excitable patient, with a fine tremor of the extended hands and moderate exophthalmos (right, 20 mm.; left, 20 mm.). There was a thyroidectomy scar; the residual left lobe was diffusely enlarged. Treat-

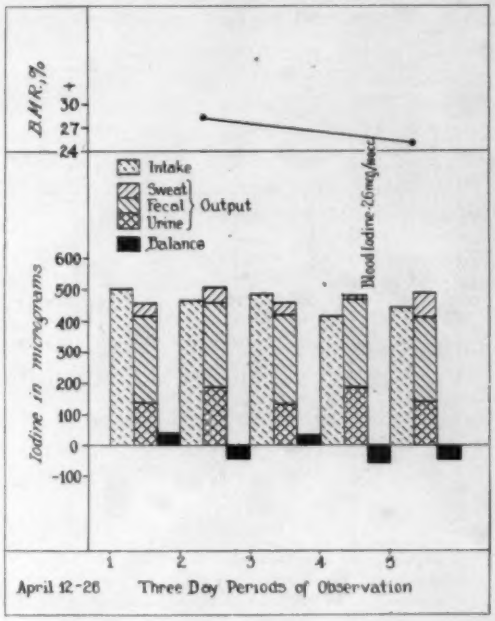

Chart 5.—Iodine balance of T. F., a woman aged 24, with exophthalmic goiter. She remained in low negative iodine balance even when maintained on sufficient iodine to keep a normal person in positive balance.

ment was by rest alone. Operation was refused by the father. The investigation of iodine and calcium balance was made from January 17 to 29.

The Wassermann and Kahn reactions of the blood were negative. The blood and urine were normal. The phenolsulfonphthalein test showed 90 per cent excretion at the end of the first hour after intravenous administration and 5 per cent excretion at the end of the second hour. The basal metabolic rate on January 17 was + 8 per cent, with the pulse rate 89, the temperature 97.8 F., the respiratory rate 19, the blood pressure 104 systolic and 50 diastolic and the body weight 119 pounds (54 Kg.). The patient was discharged January 30.

Comment.—This girl of 15 presented recurrent hyperthyroidism seven months after a right subtotal lobectomy. Immediately prior to

our investigation the basal metabolic rate was established at about + 30 per cent. This decreased to within normal limits throughout the investigation (chart 4). The blood iodine was normal on two occasions, and increased on two (chart 4). However, the symptomatic picture remained that of exophthalmic goiter. The negative iodine balance on a low intake of iodine, averaging 94 micrograms per three day period, also remained increased from two to three times over normal, averaging 309 micrograms per three day period (chart 4).

T. F., a white housewife aged 24, with typical exophthalmic goiter, was transferred April 9, 1934, to the research surgical service for this investigation and for thyroidectomy. Her protocol has been presented elsewhere.[5]

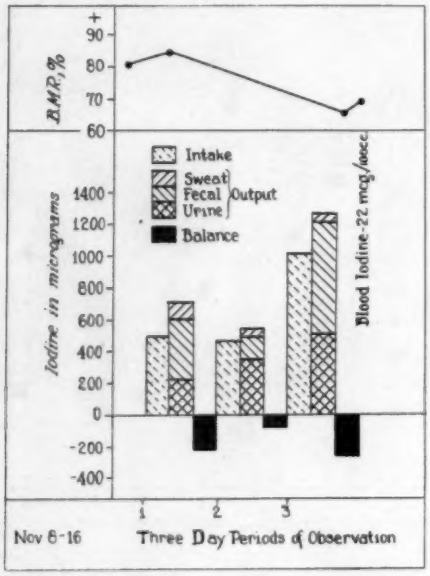

Chart 6.—Iodine balance of R. S., a woman aged 24, with exophthalmic goiter. Even with a normally sufficient intake she remained in continuous negative iodine balance.

Comments.—This patient, with a normally sufficient intake of iodine, averaging 466 micrograms per three day period, remained in low negative iodine balance, averaging 16 micrograms per three day period, over a total period of fifteen days (chart 5).[5]

R.S., a white housewife aged 24, entered the University Hospital for investigation and for thyroidectomy Oct. 30, 1934. She presented characteristic exophthalmic goiter. Her protocol is presented elsewhere.[5]

Comment.—This patient, with a normally sufficient intake of iodine, averaging 653 micrograms per three day period, remained in continuous

negative iodine balance, averaging 190 micrograms per three day period (chart 6).[5]

EFFECT OF INCREASED IODINE FEEDING ON THE IODINE BALANCE OF A NORMAL PERSON ON A LOW INTAKE OF IODINE

We have determined the effects of increased iodine feeding on the negative iodine balance of a normal man (J. R.) on a low intake of iodine.

J. R. was investigated over a period of thirty days. On 70 micrograms of iodine per three day period he remained in the continuous negative iodine balance which is typical of normal persons (table 2). The diet was then supplemented by adding milk with an increased iodine content and by giving potassium iodide in gradually increasing doses (table 2). On this increased intake of iodine, 500 micrograms daily and greater, there was immediate retention of iodine with consequently a positive iodine balance (table 2). With increased intake of iodine there ensued increased retention of iodine (table 2). The basal metabolic rate averaged —14 per cent during the fifteen days of low intake of iodine. It increased to —7 per cent during the latter nine days of increased iodine feeding (table 2).

EFFECT OF INCREASED IODINE FEEDING ON THE IODINE BALANCE OF PATIENTS WITH EXOPHTHALMIC GOITER ON A LOW INTAKE OF IODINE SIMILAR TO THAT OF OUR CONTROL (J. R.)

We have had the opportunity of determining the effect of iodine feeding on the increased negative iodine balance of 2 patients with exophthalmic goiter.

F. W., a man aged 23, was studied over a total period of sixty-one days. He presented severe exophthalmic goiter. The basal metabolic rate ranged from + 75 to + 94 per cent during our early investigation (chart 2 and table 4). On a low intake of iodine, 74 micrograms per three day period, the negative iodine balance remained continuously increased from four to five times the normal (chart 2 and table 4). The diet was then supplemented by milk with an increased iodine content and later by potassium iodide. The amounts were similar to those administered to the normal control (table 4). There was immediate tremendous retention of iodine with a consequent positive iodine balance, which was twice that of the normal person (table 4). With increases in the amount of iodine administered there ensued increases in the amount of iodine retained. Approximately 86 mg. of iodine was retained by this hyperthyroid patient prior to any appreciable change in the symptomatic picture or in the basal metabolic rate (table 4). Before operation approximately 177 mg. of iodine had been retained (table 4). The removed goiter weighed 150 Gm. It showed typical massive iodine-induced colloid involution and contained approximately 100 mg. of iodine, which is about ten times the normal for this region.

E. C., a young woman of 24, with untreated exophthalmic goiter, showed a remission of her clinical symptoms on medical management alone (table 5 and chart 3). This was accompanied by a gradual decrease of the basal metabolic

rate to within the normal limits of from + 9 to + 14 per cent (chart 3). There was an associated decrease in the excretion of iodine through all the excretory channels investigated, so that the iodine balance returned to within normal limits during the sixth three day period (chart 3). Her basal metabolic rate decreased further subsequent to administration of 10 mg. of iodine daily for a period of nine days. During the latter period there was great retention of iodine, administered as potassium iodide, with a resulting positive iodine balance (chart 3). Iodine medication was then discontinued over a period of thirty-nine days. Immediately the excretion of iodine became greater than the intake, so that the patient remained in continuous negative iodine balance during this vacation in the administration of iodine (chart 3). The basal metabolic rate also immediately increased over a period of six days to establish itself at from + 30 to + 36 per cent (chart 3). The former clinical symptoms returned.

Roentgen therapy was then instituted. This produced no immediate appreciable change in the metabolism of iodine or in the clinical status of the patient (chart 3). Iodine therapy was, therefore, reinstituted, 10 mg. of iodine as potassium iodide being given daily. Again, there was immediate tremendous retention of iodine over a period of investigation of fifteen days. This accompanied remission of the clinical symptoms and decline in the basal metabolic rate (chart 3).

EFFECT OF THYROIDECTOMY ON THE INCREASED NEGATIVE IODINE BALANCE OF EXOPHTHALMIC GOITER

Two patients with exophthalmic goiter were studied over a total period of eighteen days, one seven months after a single lobectomy, and the other two years and seven months after an adequate thyroidectomy.

The first, M. W., a girl of 15, showed recurrent hyperthyroidism seven months subsequent to a right subtotal lobectomy. Immediately prior to this investigation the basal metabolic rate was established at about + 30 per cent. This decreased to within normal limits during the period of study (chart 4). The blood iodine determination was normal on two occasions and increased on two (chart 4). However, the symptoms remained those of exophthalmic goiter. The negative iodine balance, on a low intake of iodine, averaging 94 micrograms per three day period, also remained increased to from two to three times the normal, averaging 309 micrograms per three day period. A subtotal lobectomy was done later. The removed goiter showed typical diffuse hyperplasia.

The second, T. F., a housewife of 27, showed recovery from symptoms characteristic of exophthalmic goiter two years and seven months after a bilateral subtotal thyroidectomy. The iodine balance, originally investigated over a total period of fifteen days prior to operation, had been negative, averaging 16 micrograms per three day period even with a normally adequate intake of iodine, which averaged 466 micrograms per three day period (chart 5).[5] During the present study (chart 4) the basal metabolic rate, the blood iodine and the iodine balance were normal except for a slightly increased negative iodine balance during the first three day period (chart 4). This was presumably due to menstruation.

GENERAL COMMENT

Three normal persons were thus studied as controls under various conditions over a total period of thirty-nine days. Five patients with

exophthalmic goiter were investigated over a total period of one hundred and eighty-six days. The normal persons maintained on a low intake of iodine, averaging 87 micrograms per three day period over a total period of twenty-four days, showed a continuous negative iodine balance, which averaged 126 micrograms per three day period (chart 7). The total excretion of iodine averaged 213 micrograms per three day period. The greatest excretion was through the urine, averaging 72 per cent; 15 per cent was excreted through the feces and 13 per cent through the sweat (chart 7). The normal blood iodine averaged 4.3 micrograms per hundred cubic centimeters (chart 7).

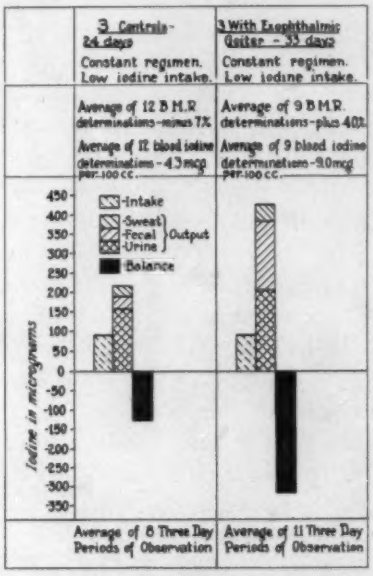

Chart 7.—A summary of the iodine balance of three normal controls (at left) compared with that of three patients with exophthalmic goiter (at right). Note that the normal persons on a low intake of iodine remained in continuous negative balance; i. e., the excretion of iodine was greater than the intake. Note also that patients with exophthalmic goiter on a low intake similar to that of the normal persons showed increased excretion of iodine particularly through the feces. This resulted in a negative iodine balance from two to three times the normal.

These data (tables 1 and 2 and charts 1 and 7) indicate that in normal persons a certain amount of iodine is excreted daily over an as yet undetermined length of time regardless of the intake of iodine. Only when iodine was furnished in excess of this amount was there a positive iodine balance (table 2). The significance of this normal negative iodine balance on a low intake of iodine needs further investigation.

Plummer[14] found that somewhat less than 0.5 mg. of thyroxin given daily maintained heat production at a normal level in a completely myxedematous patient, and he therefore concluded that the amount of thyroxine discharged by the thyroid gland daily is somewhat less than this amount. Later Boothby, Sandiford and their associates[15] showed that the daily amount of thyroxin needed to maintain normal heat production in a completely myxedematous patient was 0.25 mg.

These investigations[16] indicate that from 163 to 325 micrograms of iodine are utilized daily in the form of thyroxine. It is possible that some of the iodine resulting from the breakdown of thyroid hormone is in such form that it is not then available to the thyroid or to the tissues and that this constitutes the iodine excreted on a low intake of iodine. For our normal persons from 50 to 100 micrograms of iodine would be required daily to maintain a positive iodine balance. A comparison of Plummer's[14] and Boothby's[15] investigations with our own indicates, therefore, that normally some of this iodine resulting from the breakdown of thyroxine was daily husbanded by the body for reutilization, as in the resynthesis of thyroid hormone. Further investigation of these particular problems is necessary.

We have investigated the effect of starvation over a total period of six days (table 1) on this normal negative iodine balance. On a low intake of iodine, 98 micrograms per three day period, there was a continuous negative iodine balance throughout a preliminary period of six days. During starvation the excretion of iodine in the urine, feces and sweat continued so that a greater negative balance resulted (table 1). The excretion of iodine, however, reached a minimum on the sixth day of fast. It might have fallen still lower if the fast had been prolonged. This indicates a conservative mechanism for iodine such as was shown by von Fellenberg.[17]

The effect of increased iodine feeding on the negative iodine balance of a normal person has been shown (table 2). During a low intake of iodine, 70 micrograms per three day period for nine days, this subject showed a continuous negative iodine balance. On an increased intake of iodine, 500 micrograms daily and greater, there was immediate retention of iodine and consequently a positive iodine balance was established (table 2). With increases in the amount of iodine administered there followed increases in the amount retained (table 2). The basal metabolic rate averaged — 14 per cent during

14. Plummer, H. S.: Tr. A. Am. Physicians **31**:128, 1916; J. A. M. A. **77**: 243, 1921.

15. Boothby, W. M.; Sandiford, I.; Sandiford, K., and Slosse, J.: Ergebn. d. Physiol. **24**:728, 1925; Tr. A. Am. Physicians **40**:195, 1925. Boothby, W. M., and Baldes, E. J.: Proc. Staff Meet., Mayo Clin. **1**:166, 1926.

16. Plummer.[14] Boothby and others.[15]

17. von Fellenberg, T.: Ergebn. d. Physiol. **25**:176, 1926.

the fifteen days of low intake. It increased to — 7 per cent during the latter nine days of increased iodine feeding (table 2).

The excretion of iodine on a low intake of iodine continues even during the eighth month of gestation (table 3). In fact, during this period of increased physiologic activity the excretion of iodine may be increased.[18]

We [4a] have already presented other physiologic variables related to the metabolism of iodine and the thyroid gland. The particular part which each of these factors plays in the metabolism of iodine is still obscure.

Patients with exophthalmic goiter maintained on a low intake of iodine similar to that of normal persons showed a great increase in excretion of iodine, particularly through the feces (chart 7). This resulted in an increased negative iodine balance of from two to three times the normal. The total excretion of iodine averaged 414 micrograms per three day period. The greatest excretion occurred through the urine (chart 7), averaging 49 per cent; 40 per cent was excreted through the feces and 11 per cent through the sweat. Two other patients with exophthalmic goiter (charts 5 and 6) were maintained on an intake of iodine sufficient to keep a normal patient in positive iodine balance. However, these 2 patients also showed a negative iodine balance.

The blood iodine was increased in the patients with exophthalmic goiter, averaging 9 micrograms per hundred cubic centimeters (chart 7). Some have theorized that this increase in blood iodine in hyperthyroidism follows increased retention of iodine, due particularly to insufficiency of the excretory function of the biliary system.[19] The excretion of iodine by the biliary system is important in the metabolism of iodine, as has been emphasized by Elmer and Luczynski,[20] De Courcy,[19] Maruno [21] and others.

A moderately increased blood iodine has been observed in certain patients presenting various diseases of the biliary system.[22] The relationship between the liver and the thyroid gland [23] and exophthalmic goiter [24] has been investigated.[25] However, our present data (chart 7) indicate that the increased blood iodine in hyperthyroidism may result from augmentation of the total iodine metabolism. There are an

18. Puppel, I. D.; Ross, T. F., and Curtis, G. M.: Unpublished data.

19. DeCourcy, J. L.: West. J. Surg. **45**:432, 1937.

20. Elmer, A. W., and Luczynski, Z.: Compt. rend. Soc. de biol. (*a*) **114**: 351 and (*b*) 1340, 1933; (*c*) **115**:647 and (*d*) 1717, 1934.

21. Maruno, Y.: Jap. J. Gastroenterol. **3**:97, 111 and 123, 1931.

22. Bierbaum, R.; Puppel, I. D., and Curtis, G. M.: Unpublished data.

23. Doetsch, H.: Biochem. Ztschr. **279**:233, 1935. Repetto, E.: Arch. ital. di chir. **40**:564, 1935.

24. Weller, C. V.: Tr. A. Am. Physicians **45**:71, 1930.

25. Puppel, I. D., and Curtis, G. M.: Unpublished data.

increase in its mobilization, an increase in its circulation and an increase in its excretion through one or all excretory channels. This results in a profound disturbance of the iodine balance (chart 7).

The true nature of the increased negative iodine balance in exophthalmic goiter is not clear. Presumably it results from an increased consumption of thyroid hormone and a consequent increased mobilization and elimination of iodine. This in the presence of an intake of iodine which is not only insufficient to meet the increased requirements of hyperthyroidism but even insufficient to meet the normal requirements for iodine (chart 7) results in the increased negative iodine balance.

We have already demonstrated that a patient with hypothyroidism who was maintained on desiccated thyroid for months showed increased excretion of iodine through all channels. This resulted principally from excretion of the amino acid iodine [26] and to a lesser extent from that of the inorganic iodine [27] which exists in desiccated thyroid. However, the intake of iodine was also increased by ingestion of desiccated thyroid so that the iodine balance remained physiologic. Thyroid therapy was then discontinued. Immediately there ensued an increased negative iodine balance which simulated that of hyperthyroidism. This presumably resulted from continued consumption of stored thyroid hormone and consequent continued mobilization and excretion of iodine in the presence of the lessened intake of iodine. As the stored thyroid hormone of this hypothyroid patient was depleted, the excretion of iodine slowly decreased from 1,250 to 200 micrograms per three day period over a period of nine days following cessation of the ingestion of desiccated thyroid. The iodine balance again returned to within physiologic limits. This was accompanied by a decrease in the basal metabolic rate of from —4 to —20 per cent.

Elmer and Luczynski [20a, c] showed experimentally that subsequent to gastric administration or intravenous injection of thyroxin from 90 to 93 per cent of the iodine excreted in the bile and from 49 to 63 per cent of the iodine excreted in the urine is in the form of split products of thyroxin. In addition, Asimoff and Estrin [28] recently found that after feeding dried thyroid gland to dogs and after intravenous injection of thyroxin the amount of iodine excreted in the urine was increased. This urine in no instance affected metamorphosis of the

26. (*a*) Harington, C. R., and Randall, S. S.: Biochem. J. **23**:373, 1929. (*b*) Foster, G. L.; Gutman, A. B., and Gutman, E. B.: Proc. Soc. Exper. Biol. & Med. **30**:1028, 1933. (*c*) Abelin, I.: Schweiz. med. Wchnschr. **65**:872, 1935. (*d*) Thompson, W. O.; McLellan, L. L.; Thompson, P. K., and Dickie, L.: J. Clin. Investigation **12**:235, 1933.

27. Foster and others.[26b] Abelin.[26c]

28. Asimoff, G., and Estrin, E.: Ztschr. f. d. ges. exper. Med. **76**:380 and 399, 1931.

axolotl. It seems, therefore, that with increased utilization of thyroid hormone there ensues increased elimination of iodine. This in the presence of an intake of iodine similar to that of normal persons results in an increased negative iodine balance.

There are several factors which may influence this increase in the negative iodine balance in exophthalmic goiter. We have studied the effect of certain of the more common types of treatment (charts 3 and 4, and table 4). On medical management alone, including rest in bed, a diet high in calories and calcium therapy (chart 3), a patient with untreated exophthalmic goiter showed remission of the clinical symptoms and decrease of the basal metabolic rate to within normal limits. There was associated decrease in the excretion of iodine through all excretory channels investigated, so that the iodine balance returned to within normal limits during the sixth three day period (chart 3). Her basal metabolic rate decreased further subsequent to administraion of 10 mg. of iodine daily for a period of nine days. During the latter period there was great retention of iodine (chart 3). Iodine medication was then discontinued over a period of thirty-nine days. Immediately there ensued greater excretion than intake of iodine, so that the patient remained in continuous negative iodine balance during this period of vacation in medication with iodine (chart 3). There is, therefore, an inescapable possibility that the increased negative iodine balance of the patient with exophthalmic goiter results, at least in certain cases, from previous iodine feeding and subsequent high storage of easily mobilizable iodine within the body. This effect is definitely demonstrated by E. C. (chart 3). The old symptoms and the increased basal metabolic rate also reappeared. Massive roentgen therapy produced no appreciable immediate change in the metabolism of iodine (chart 3) or in the clinical status. Iodine therapy was, therefore, reinstituted with 10 mg. of iodine as potassium iodide daily. Again there ensued immediate tremendous retention of iodine over a period of fifteen days. This accompanied a second remission of the clinical symptoms and a decrease of the basal metabolic rate (chart 3).

Another patient (table 4) showed the typical increased negative iodine balance of exophthalmic goiter when maintained on a low intake of iodine. The diet was then supplemented with milk containing an increased content of iodine and later with potassium iodide. The doses were similar to those administered to a normal person (compare tables 2 and 4). Immediately there was great retention of iodine with consequently a positive iodine balance. This was twice that of the normal person (compare tables 2 and 4). Hyperthyroid symptoms and an increased basal metabolic rate persisted even though there was a positive iodine balance.

Boothby [2b] and Boothby and Sandiford [2c] showed that a negative nitrogen balance is not necessarily characteristic of exophthalmic goiter.

By carefully controlled experiments, they demonstrated that nitrogen equilibrium can be readily maintained if the dietary intake is sufficiently large to meet the body's requirements. This holds true for the metabolism of carbohydrate and fat as well as for that of protein, so that the patient with exophthalmic goiter does not necessarily lose weight. These observations have been confirmed repeatedly. We now demonstrate that an increased negative iodine balance is not necessarily characteristic of exophthalmic goiter. The data on the patients (F. W. and E. C.) show that iodine equilibrium and even a positive iodine balance can be readily maintained if the intake of iodine is sufficiently large or in excess of the requirements of the hyperthyroid organism (table 4 and chart 3). This is also true for the calcium balance.[29] In all instances of the latter the augmentation of the endogenous metabolism in hyperthyroidism is then masked by the increased ingestion of the necessary foodstuffs even to several times normal.

The immediate great retention of iodine, from two to three times normal, which may occur subsequent to administration of iodine (compare table 2 with table 4 and chart 3) indicates repletion of the depleted iodine stores of the patient with exophthalmic goiter as discussed in our previous paper.[4b]

This great retention of iodine may also indicate a greater than normal capacity for storing iodine in the diffusely hyperplastic thyroid of the patient with exophthalmic goiter in the presence of large amounts of iodine. Approximately 86 mg. of iodine was retained by one hyperthyroid patient (table 4) prior to any appreciable change in the clinical symptoms or in the basal metabolic rate. Before operation approximately 177 mg. of iodine had been retained by this patient (table 4). The removed goiter weighed 150 Gm. It showed typical massive iodine-induced colloid involution and contained approximately 100 mg. of iodine, which is about ten times the normal for this goitrous region.

This patient (table 4), who received 48 mg. of iodine daily preoperatively and postoperatively, also showed an increase in the excretion of iodine immediately following thyroidectomy.

A patient (chart 4) with recurrent hyperthyroidism seven months after a single lobectomy revealed an increased negative iodine balance characteristic of exophthalmic goiter. Another patient (chart 4) showed complete recovery from symptoms characteristic of exophthalmic goiter two years and seven months after adequate subtotal thyroidectomy. At this time the iodine balance was normal. However, previous to operation investigation had shown a negative iodine balance even with a normally adequate intake of iodine (chart 5). Similarly, a patient with nodular goiter and hyperthyroidism showed a dramatic

29. Puppel, I. D.; Klassen, K. P., and Curtis, G. M.: Unpublished data.

fall in excreted iodine to within normal limits as early as the sixth to twelfth day after thyroidectomy.[25] This was accompanied by clinical improvement. The basal metabolic rate and the blood iodine had also returned to within normal limits.[25] Another patient with toxic nodular goiter showed similar findings.[25] It is thus established definitely that the increased utilization of thyroid hormone augments the mobilization, circulation and excretion of iodine in hyperthyroidism. Adequate thyroidectomy dramatically abolishes these profound changes in the metabolism of iodine.

There is an increased negative iodine balance in diseases of the thyroid gland other than exophthalmic goiter. Two patients presenting toxic nodular goiter with a basal metabolic rate averaging + 30 per cent showed an increase in negative iodine balance over normal. There was a return to normal following adequate subtotal thyroidectomy.[25]

A patient with carcinoma of the thyroid with pulmonary metastases and euthyroidism also showed an increase in negative iodine balance over normal.[25] However, not all patients with goiter show an increased negative iodine balance, as was evidenced by an investigation of two patients with nontoxic nodular goiter over a total period of thirty-six days.[25] One (D. W.) revealed a physiologic iodine balance. The other (B. S.) even showed a tendency toward more than normal retention of iodine.[25]

There may be causes of an increased negative iodine balance other than clinical hyperthyroidism. One patient with postoperative hypoparathyroidism who showed increased retention of calcium seven months after a subtotal thyroidectomy revealed an accompanying increase in excretion of iodine with a resulting increase in negative iodine balance.[25] One patient (chart 4) showed a slight increase in negative iodine balance, which presumably accompanied menstruation. A woman eight months pregnant also showed an increase in negative iodine balance.[18] Also, immediately subsequent to discontinuation of thyroid therapy in a case of hypothyroidism there continued to be temporarily increased excretion of iodine and increased negative iodine balance.[25] Immediately subsequent to extrathyroid surgical intervention there resulted a great increase in negative iodine balance (table 1).

There is slowly accumulating evidence that there may be several factors, such as the metabolism of other minerals,[1b] the influence of other hormones[30] and endocrine glands,[31] emotional reactions,[17] the

30. Houssay, B. A.; Mazzocco, P., and Biasotti, A.: Compt. rend. Soc. de biol. **111**:82, 1932. Schockaert, J. A., and Foster, G. L.: J. Biol. Chem. **95**: 89, 1932. Closs, K.; Loeb, L., and MacKay, E.: ibid. **96**:585, 1932. Stimmel, B. F.; McCullagh, D. R., and Picha, V.: J. Pharmacol. & Exper. Therap. **57**:49, 1936.

31. Houssay, B. A.: New England J. Med. **214**:961, 1936. Marine, D.: Am. J. M. Sc. **180**:767, 1930. Black, E.; Hupper, M., and Rogers, J.: Am. J. Physiol. **59**:222, 1922.

state of the autonomic nervous system,[32] infection,[33] the supply of vitamins,[34] production of an antithyrotropic protection substance,[35] the intake, absorption and excretion of iodine, and the formation of thyroid colloid, which cause a deviation from, or are concerned with, the maintenance of normal function of the thyroid and normal metabolism of iodine. The relation of these factors to the pathogenesis of exophthalmic goiter remains obscure. Further investigation of these particular problems concerned with the metabolism of iodine should prove valuable.

COMMENT

1. Three normal persons maintained on a low intake of iodine, averaging 87 micrograms per three day period over a total period of twenty-four days, remained in continuous negative iodine balance, which averaged 126 micrograms per three day period. The total excretion of iodine averaged 213 micrograms per three day period. The greatest excretion was through the urine, averaging 72 per cent; 15 per cent was excreted through the feces and 13 per cent through the sweat. The normal blood iodine averaged 4.3 micrograms per hundred cubic centimeters. These data indicate that in normal persons a certain amount of iodine is excreted daily over an as yet undetermined length of time regardless of the amount of iodine taken in. Only when iodine was furnished in excess of the amount excreted daily was there a positive iodine balance.

2. The significance of this negative iodine balance of normal persons on a very low intake of iodine is not clear. It is possible that some of the iodine resulting from the breakdown of thyroid hormone is in such form that it is not then available to the thyroid or to the tissues and that this constitutes iodine excreted on a low intake of iodine. The effect of starvation, of iodine feeding, of pregnancy and of extrathyroid surgical operation on this negative iodine balance of normal persons was included in the study.

3. Three patients with exophthalmic goiter maintained on a low intake of iodine similar to that of the normal persons showed a great increase in the excretion of iodine, particularly through the feces. This resulted in an increased negative iodine balance of two to three times

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the normal. The total excretion of iodine averaged 414 micrograms per three day period. The greatest excretion occurred through the urine, averaging 49 per cent; 40 per cent was excreted through the feces and 11 per cent through the sweat.

4. Two other patients with exophthalmic goiter were maintained on an intake of iodine sufficient to keep a normal person in positive iodine balance. These 2 patients also showed a negative iodine balance.

5. The blood iodine of patients with exophthalmic goiter was increased, averaging 9 micrograms per hundred cubic centimeters. Some have believed that this increase in the blood iodine of patients with hyperthyroidism results from retention of iodine by the body as a result of hepatic insufficiency. However, the present data indicate that the increased blood iodine in such patients may result from augmentation of the total iodine metabolism. There is an increase in mobilization of iodine, an increase in circulation of iodine and an increase in excretion of iodine through one or all excretory channels. This results in profound disturbance of the iodine balance.

6. The true nature of the increased negative iodine balance of exophthalmic goiter is not clear. Presumably it results from increased consumption of thyroid hormone and consequent increased mobilization and elimination of iodine. This in the presence of an intake of iodine which is not only insufficient to meet the increased requirements of hyperthyroidism but even insufficient to meet the normal requirements for iodine results in the increased negative iodine balance. There is an inescapable possibility that it results, at least in certain cases, from previous iodine feeding and the subsequent high storage of easily mobilizable iodine within the body. The true significance of this increased excretion of iodine in exophthalmic goiter in the presence of an apparent need for iodine remains unknown. This chemical manifestation of the disease becomes still more significant when it is realized that the diffusely hyperplastic thyroid gland retains its natural avidity for iodine.

7. There are several factors which may influence the increased negative iodine balance in exophthalmic goiter. Investigations of the effects of general hospital management, which included rest in bed, a diet high in calories and calcium therapy, of iodine feeding, of roentgen therapy and of thyroidectomy were included in the study.

8. The increased negative iodine balance in hyperthyroidism returned to within normal limits subsequent to adequate thyroidectomy.

9. Increased feeding of iodine to a normal person on a low intake of iodine produced an immediate positive iodine balance. Similar increased feeding of iodine to a patient with hyperthyroidism resulted in immediate tremendous retention of iodine and a consequent positive iodine balance, which was twice that of the normal.

10. A negative iodine balance is not necessarily characteristic of exophthalmic goiter. Even a positive iodine balance can be readily maintained if the intake of iodine is sufficiently large or in excess of the increased requirements of the hyperthyroid organism.

11. There is an increased negative iodine balance in persons with toxic nodular goiter. In persons with nontoxic nodular goiter the iodine balance is physiologic.

12. It is not certain whether the incipient thyroid hyperplasia in hyperthyroidism precedes or follows the onset of this process of increased mobilization, utilization and excretion of iodine. However, it is possible that the thyroid hyperplasia in hyperthyroidism is secondary to the increased loss of body iodine and that it is compensatory in an attempt to meet continued increased demand for utilizable iodine subsequent to consumption of the available stores.

13. Causes of an increased negative iodine balance other than clinical hyperthyroidism were included in the study. The relation of these other factors to the pathogenesis of exophthalmic goiter remains obscure. Further investigation of these particular problems concerned with the metabolism of iodine should prove valuable in throwing more light on the true nature of this disease.

SUMMARY

Three normal persons maintained on a low intake of iodine remained in continuous negative iodine balance. The blood iodine averaged 4.3 micrograms per hundred cubic centimeters. There are several variants related to the iodine balance of normal persons. The effects of starvation, of iodine feeding, of pregnancy and of extrathyroid surgical treatment on this negative iodine balance of normal persons on a low intake of iodine are considered in this report.

Three patients with exophthalmic goiter maintained on a low intake of iodine similar to that of the normal persons showed a great increase in the excretion of iodine, particularly through the feces. This resulted in an increase in negative iodine balance of from two to three times the normal. The blood iodine was increased, averaging 9 micrograms per hundred cubic centimeters. There are several factors which may influence the increased negative iodine balance of patients with exophthalmic goiter on a low intake of iodine. Investigations of the effect of general hospital management, which included rest in bed, a diet high in calories and calcium therapy, and of iodine feeding, of roentgenotherapy and of thyroidectomy are presented.

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EXPERIMENTAL CARBON TETRACHLORIDE POISONING IN THE CAT

II. THE INFLUENCE OF LIGATION OF SINGLE BILE DUCTS

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Little information is available regarding the influence of obstruction of bile ducts on the response of the liver to hepatoxic agents. The only detailed experimental data bearing directly on this problem that we have encountered are those presented by Love,[1] who investigated the effect of phosphorus on the livers of rats with obstruction of the common bile duct. The present study consists of an investigation of the effect of ligation of single bile ducts on the hepatic lesions produced by carbon tetrachloride. The cat was employed because an extensive experience with experimental lesions of the biliary tract and liver in this animal[2] had yielded material which might be used as a basis for comparison in certain phases of the study.

MATERIAL AND METHODS

Forty-four adult cats weighing from 1.2 to 3.6 Kg. were employed in this study. They were divided into two groups and maintained on a diet of fresh raw scrap meat and milk. In group 1 (27 cats) each animal received a subcutaneous injection of chemically pure carbon tetrachloride in the dosage of 0.3 cc. per kilogram of body weight, with the exception of 4 animals each of which received 0.25 cc. per kilogram. At varying intervals (from one to twenty-five days) after the administration of carbon tetrachloride in different cases, estimations were made

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of the degree of bromsulphalein retention and of the concentration of bilirubin in the serum and of nonprotein nitrogen in the blood. In all but a few instances 5 cc. of india ink diluted 1:4 in physiologic solution of sodium chloride was then injected intravenously, and after five minutes the animals were killed by bleeding under light ether anesthesia. At autopsy, in the majority of instances urine was aspirated from the bladder and was examined for urobilinogen, albumin and formed elements. Nine of this group died spontaneously (in from two to four days). The reported observations were made on 18 surviving animals.

In each animal of group 2 (17 cats), under light anesthesia one or more ducts leading from the left lobes of the liver were doubly ligated and divided. The exact site of obstruction was determined at autopsy. Seven animals in this group died spontaneously within from four to nine days after operation. Fourteen days after ligation of the ducts each of the surviving animals (10) received subcutaneously 0.3 cc. of chemically pure carbon tetrachloride per kilogram of body weight. They were treated subsequently in the same manner as group 1, being put to death from one to fourteen days after being given the injection. At necropsy, 2 were rejected as unsatisfactory, one because of complicating suppurative cholangitis, the other because of reconstruction of the obstructed bile duct or ducts. The observations reported here were made on the remaining animals (8) in this group.

Tissues were fixed in a solution of formaldehyde (1:10) or in Zenker's solution in which formaldehyde was substituted for acetic acid. Some were subsequently frozen, cut and stained with nile blue sulfate and scarlet red; the remainder were blocked in paraffin, cut, and stained with hematoxylin and eosin and Mallory's stain for connective tissue. Frozen sections (from 8 to 25 microns thick) were examined under crossed Nicol prisms for doubly refractile material.

OBSERVATIONS IN GROUP 1 (CATS 1 TO 18)

The findings in this group of animals have been reported in detail elsewhere.[2a] They may be summarized as follows:

After the subcutaneous injection of 0.3 cc. of carbon tetrachloride per kilogram of body weight, congestion, hemorrhage, and degeneration and necrosis of hepatic cells in the central zones of the hepatic lobules reached a maximum at about forty-eight hours. At this time, about two thirds of each lobule might be involved. The necrotic débris was rapidly cleared away, practically disappearing by the sixth day, leaving a relatively small collapsed reticulated central area consisting of the mesenchymal supporting stroma and sinusoids with a number of yellow-pigmented phagocytic and inflammatory cells.

Evidence of regeneration and repair of the hepatic cells was observed at a very early stage. At forty-eight hours mitotic figures were present in cells immediately bordering the central necrotic zones and were seen in this situation and also in the central zones themselves as late as the fourteenth day. At forty-eight hours an atypical form of newly regenerated hepatic epithelium, flattened, hyperchromatic, appeared around the necrotic zones and subsequently invaded and compressed the latter until, at ten days, these zones were completely filled with these atypical cells.

The normal arrangement of the hepatic cells in cords in the central zones began to reappear at the tenth day. On the twelfth day the great majority of the lobules appeared essentially normal except for the presence of the new flattened hyperchromatic cells in their interiors; at fourteen days there were fewer of these cells and correspondingly more normal polygonal hepatic cells, many of which contained mitotic figures.

During the third and fourth weeks after injection of carbon tetrachloride there appeared to be a secondary recrudescence of regressive phenomena. At sixteen days the liver resembled that at six days, with more central congestion. Extensive degeneration and necrosis of hepatic cells were seen at twenty-one days, the lesion being most marked in the intermediate zones of the lobules. The atypical flattened, newly regenerated cells in the central zone were involved to only a relatively slight degree. At twenty-five days about one half of each lobule was involved in the degenerative process, which was now most marked in the central zone of the lobule, which contained many atypical cells with no normal cord arrangement and many inflammatory and red blood cells. No mitotic figures were seen during this period of secondary regressive phenomena (at from sixteen to twenty-five days).

Except in 2 animals examined at twenty-four and forty-eight hours, little or no india ink was seen in the Kupffer cells during the first six days after the injection of carbon tetrachloride. Those cells which were active in this regard were confined to the relatively normal peripheral zones of the lobules. Subsequently, from the eighth to the twenty-fifth day, the Kupffer cells throughout the lobules—usually, however, chiefly at the peripheries—were very large and contained large amounts of ink.

Although there was extensive vacuolation of hepatic cells in the intermediate zones (midzones) of the lobules, stainable lipid was most abundant in the central degenerated zones twenty-four hours after injection and in the peripheral zones after the sixth day. Little or no stainable lipid was seen in the Kupffer cells. Doubly refractile material varied considerably in amount and distribution throughout the experimental period but was rather abundant in the hepatic cells and practically absent from the Kupffer cells during the first six days.

Hyperbilirubinemia (from 0.2 to 1.76 mg. per hundred cubic centimeters) and bromsulphalein retention (from 20 to 100 per cent) were present in every case in the first six days. Excessive urobilinuria was also present during this period. The highest values for serum bilirubin, 1.6 and 1.76 mg. per hundred cubic centimeters, were obtained on the fourth day. No hepatic functional impairment was demonstrable by these methods after the sixth day in this series.

OBSERVATIONS IN GROUP 2 (CATS 19 TO 26)

As stated previously, all animals in this group were subjected to ligation of single hepatic ducts fourteen days prior to the injection of carbon tetrachloride. The time stated in each case represents the time elapsing since the injection.

One Day (Cat 19).—Obstructed Lobules: Changes were present characteristic of biliary stasis of fifteen days' duration.[2b] There was white bile in the dilated obstructed ducts from the left lobe of the liver. There was slight sporadic degeneration, with no sharp delimitation of the central zones. Kupffer cells were exceedingly numerous, particularly in the peripheral zones, and contained large amounts of ink. Nonobstructed Lobules: The Kupffer cells were slightly smaller and less numerous than in the obstructed lobules. Otherwise, except for the changes characteristic of biliary stasis in the latter, both portions of the liver presented a similar appearance.

Two Days (Cat 20).—There was white bile in the dilated obstructed ducts from the left lobe, with sharp gross demarcation between obstructed and nonobstructed portions of the liver. The former showed grossly accentuated markings, a smoother surface and smaller lobules. The latter showed indistinct markings and a finely pitted surface, with depression of the centers of the lobules. Obstructed Lobules: Changes were present characteristic of biliary stasis of sixteen days' duration. There was marked congestion of the inner half of each

lobule, with hepatic cells undergoing degeneration and containing hyaline droplets, especially in the periphery of the central degenerated zone. There was little vacuolation but a rather marked infiltration by inflammatory cells about the central veins. There were enormous numbers of large Kupffer cells containing large quantities of india ink, especially in the peripheral zone of each lobule. Nonobstructed Lobules: There was less central congestion than in obstructed lobules. An intense hyaline change was present in the central degenerated zone of each lobule, which was well separated from the peripheral viable zone by an intermediate area of marked degeneration and disintegration of hepatic cells. There were many large empty spaces in this zone. The cell cords were jumbled in the interior and were well preserved at the periphery. There were many Kupffer cells containing ink, fewer, however, than in obstructed lobules. The unobstructed portions showed distinctly more damage than the obstructed portions.

Three Days (Cat 21).—The gross appearance was similar to that of cat 20 (two days). Obstructed Lobules: Changes were present characteristic of biliary stasis of seventeen days' duration. The central zones were rather small, moderately congested and degenerated, and contained few yellow-pigmented cells and several patches of viable hepatic cells, many of which were very large and apparently newly regenerated. There was extensive vacuolation. Many mitotic figures were seen in all portions of the lobules. There was moderate infiltration by inflammatory cells about the central veins, and several large clear spaces were present between the sinusoids. The sinusoids showed little tendency to collapse as in uncomplicated carbon tetrachloride intoxication. Scattered throughout the lobules were many Kupffer cells filled with ink. Nonobstructed Lobules: The Kupffer cells were fewer than in the obstructed portions. Except for the changes due to stasis in the latter, the two portions of the liver appeared identical.

Four Days (Cat 22).—The gross appearance was similar to that of cat 20 (two days). Obstructed Lobules: There was little degeneration or vacuolation. The central zones were still demarcated from the peripheral zones. They contained occasional residual degenerated hepatic and inflammatory cells and were filled with hyperchromatic, apparently newly regenerated hepatic cells with large and at times multiple nuclei. There was no regular cord arrangement of the hepatic cells in the central zones. There were many Kupffer cells filled with ink, chiefly in the peripheral zones. Nonobstructed Lobules: Fewer Kupffer cells were seen. Otherwise, apart from lesions of biliary stasis, the two portions of the liver appeared identical.

Six Days (Cat 23).—The gross changes were similar to those of cat 20 (two days). Except for changes characteristic of biliary stasis in the obstructed lobules and extensive vacuolation throughout, the liver appeared essentially normal microscopically, with no difference between the obstructed and nonobstructed portions. There were numerous Kupffer cells containing large amounts of ink.

Eight Days (Cat 24).—The gross appearance was similar to that of cat 20 (two days). Obstructed Lobules: There were changes characteristic of biliary stasis of twenty-two days' duration. Otherwise the majority of the lobules appeared normal. A few showed slight congestion and slight infiltration by inflammatory cells in the central zones. There were numerous large Kupffer cells containing large amounts of ink. No mitotic figures were seen. Nonobstructed Lobules: There were moderate central degeneration and necrosis (inner one third to one half), with considerable congestion and infiltration by inflammatory cells. There was considerable disintegration with disappearance of hepatic cells in the central zone, also dilatation and collapse of the sinusoids. Many atypical new hepatic

cells were present, and mitotic figures were seen in occasional cells in the intermediate zone. There were fewer Kupffer cells than in the obstructed lobules and distinctly more damage than in the latter.

Ten Days (Cat 25).—There was white bile in the dilated obstructed ducts from the left lobes, with a sharp line of demarcation between obstructed and nonobstructed portions of the liver, the entire surface of which was smooth. Except for changes characteristic of stasis in the obstructed lobules, the entire liver appeared essentially normal histologically, and there was no other difference between the two portions of the organ. There were enormous numbers of large Kupffer cells containing large amounts of ink.

Fourteen Days (Cat 26).—White bile was observed in the dilated obstructed ducts from the left lobes, and a sharp line of demarcation between the obstructed and the nonobstructed portions of the liver. The surface of the liver was pitted, and there were small central lobular depressions. Except for changes typical of biliary stasis in the obstructed lobules, the two portions of the liver showed similar changes. Marked degeneration and necrosis were present in the inner thirds of the lobules, with congestion and inflammatory cell infiltration of the central zones, which contained many large yellow-pigmented cells and atypical new hepatic cells. No mitotic figures were seen; there were relatively few Kupffer cells, and these contained little ink. The regressive changes were more marked than at any time since the second day in this series.

Stainable Lipid and Doubly Refractile Material.—The amount and distribution of stainable lipid and doubly refractile material in the liver are indicated in table 1.

Functional Findings.—The concentration of bilirubin in the serum and of nonprotein nitrogen in the blood and the degree of urobilirubinuria and of bromsulfonphthalein retention are presented in table 2.

Summary of Observations in Group 2.—Changes typical of biliary stasis in a single lobe of from fourteen to twenty-eight days' duration were present in the obstructed portions of the liver of each cat, not differing significantly from those in animals not receiving carbon tetrachloride.[2b]

Congestion and degeneration and necrosis of hepatic cells in the central zones of the lobules of the obstructed lobes reached a maximum at forty-eight hours, at which time the inner third of each of these lobules was involved in the regressive changes. There was relatively little disintegration of hepatic cells at any time, the central zones of these lobules being rapidly filled by newly formed cells until, at six days, the obstructed lobules presented an appearance which is characteristic of stasis of a single lobe of corresponding duration except for slight infiltration by inflammatory cells. This "normal" appearance persisted until fourteen days, at which time there was recrudescence of regressive phenomena and congestion, involving the inner thirds of the lobules, of approximately the same severity as observed at forty-eight hours.

Mitotic figures were seen only on the third day, but repair of the damaged central zones, beginning at three days, was effected rapidly through their invasion by newly formed, various-sized hyperchromatic

atypical hepatic cells. At six days the lobules appeared "normal," the regular cord arrangement of the hepatic cells having been restored. At this time the hepatic cells themselves appeared normal. At from one to ten days each obstructed lobule contained large numbers of Kupffer cells, which were generally of enormous size and filled with ink. They were usually scattered throughout the lobule but were often most abundant in the peripheral zone. At fourteen days, at the time of the recrudescence of the regressive phenomena, there were relatively few recognizable Kupffer cells, and these contained little ink.

TABLE 1.—*Stainable Lipid and Doubly Refractile Material*

		Stainable Lipid				Double Refractile Material			
		Nonobstructed Lobules		Obstructed Lobules		Nonobstructed Lobules		Obstructed Lobules	
Day	Cat	Hepatic Cells*	Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells	Kupffer Cells
1	34	±	±	±	±	±	2	±	2
2	35	2 (p)	±	4 (c)	±	±	3 (c)	±	3 (c)
3	36	..	..	4 (c)	±	±	4 (p)	2 (c)	±
4	37	4	0	4	0	±	3	±	3
6	38	4 (p)	2	4 (p)	2	±	4 (l)	±	4 (l)
8	39	4	±	4	±	±	3	±	2 (l)
10	40	4	±	4	±	±	±	±	±
14	41	4 (p)	±	4 (p)	±	±	±	±	±

* In this and the following columns the letters following the grades given mean that the material was (p) confined chiefly to the peripheral zones of the lobules, (c) confined chiefly to the central zones of the lobules, and (l) confined chiefly to the intermediate zones of the lobules.

TABLE 2.—*Functional Effects*

Cat	Days	Serum Bilirubin, Mg. per 100 Cc.	Bromsulphalein Retention, Percentage	Urine Urobilinogen Concentration	Blood Nonprotein Nitrogen, Mg. per 100 Cc.
34	1	0.3	40	1:100	39
35	2	0.3	100	1:200	49
36	3	0.0	0	1:20	42
37	4	0.0	0	1:30	36
38	6	0.5	50	1:100	31
39	8	0.3	10	1:80	..
40	10	0.0	0	1:10	..
41	14	0.3	0	1:40	35

Except at two and eight days, the regressive changes were of approximately equal severity in obstructed and nonobstructed portions of the liver. At these stages the damage was distinctly more marked in the latter. This difference was particularly striking at eight days, at which time the obstructed lobules showed only the lesions characteristic of biliary stasis, while the nonobstructed lobules showed moderate degeneration and necrosis of hepatic cells and congestion, with considerable disintegration of hepatic cells in the central zones. Except for the presence of a few mitotic figures and many newly formed atypical hepatic cells at eight days in the nonobstructed lobules,

these presented essentially the same evidences of regeneration and repair as did the obstructed lobules at each stage of the experiment. In practically every instance the Kupffer cells in the nonobstructed lobules were smaller and fewer and contained less ink than those in the obstructed lobules. There were no significant differences between the two portions of the liver in regard to the quantity and distribution of stainable lipid and doubly refractile material.

COMPARISON OF OBSERVATIONS IN ANIMALS WITH AND WITHOUT OBSTRUCTION OF SINGLE DUCTS

During the first six days after injection of carbon tetrachloride the changes in the livers of cats subjected to ligation of single ducts were much less marked than those in the livers of cats with uncomplicated carbon tetrachloride intoxication.[2a] During this period, in the animals with stasis of bile in a single lobe of the liver the characteristic features of carbon tetrachloride intoxication, including central congestion of lobules, inflammatory cell infiltration, degeneration and necrosis of hepatic cells, with subsequent regeneration and repair, were in no instance pronounced. The most striking difference was noted on the fourth day, at which time in uncomplicated carbon tetrachloride intoxication the central zones of lobules had been largely cleared of débris of disintegrated hepatic cells and were markedly compressed, contained numerous inflammatory and phagocytic cells and were extensively invaded by newly formed atypical hyperchromatic hepatic cells growing in from the periphery. In the animals with stasis of bile in a single lobe of the liver the central zones of the lobules at this stage, although they did not present the normal cordlike arrangement of hepatic cells, showed comparatively little evidence of cellular degeneration, necrosis, disintegration, atrophy or compression. At six days the liver appeared essentially "normal" in these animals, whereas in those with uncomplicated carbon tetrachloride intoxication it showed evidence of extensive residual damage.

Another striking point of difference lay in the fact that in the group with obstruction of single ducts there was visible throughout the obstructed lobules an abundance of large Kupffer cells filled with ink at all stages of the experiment except at fourteen days, when there was an apparent recrudescence of regressive phenomena. Except in 2 animals examined at one and two days in the series with uncomplicated carbon tetrachloride intoxication, the Kupffer cells contained little or no ink during the first six days. What few Kupffer cells were apparently functioning actively in this respect were located peripherally in the lobules. After the sixth day large numbers of ink-laden Kupffer cells were present in these animals also. It may be of significance that during the first six days doubly refractile material was rather abundant

in the Kupffer cells and practically absent from the hepatic cells in the group with obstructed ducts, in contrast to an almost exact reversal of this distribution in the group with uncomplicated carbon tetrachloride intoxication. It is also interesting to note that the distribution of both stainable lipid and doubly refractile material in obstructed and nonobstructed lobules of the animals in group 2 was essentially the same as that in animals subjected to single duct ligation alone.[2b]

The morphologic differences between the two groups of animals are reflected in the functional findings. Particularly striking is the fact that whereas in the animals with uncomplicated carbon tetrachloride intoxication the concentration of bilirubin in the serum reached a maximum of from 0.8 to 1.76 mg. per hundred cubic centimeters at three and four days, with bromsulphalein retention of from 40 to 100 per cent, the animals with obstruction of single ducts showed no retention of bilirubin or dye at that stage. It is noteworthy that slightly abnormal functional activity was demonstrated at eight days, when the nonobstructed lobules were moderately damaged, and at fourteen days, when there was a recrudescence of regressive phenomena in the central zones. Normal function in these respects was demonstrated in the animals with uncomplicated carbon tetrachloride intoxication during the period of recrudescence of regressive phenomena (at from sixteen to twenty-five days).

COMMENT

On the basis of these observations two conclusions appear to be justified: 1. The hepatotoxic effect of carbon tetrachloride is less marked when a portion of the liver has been placed in stasis by ligation of one or more single hepatic ducts. 2. Under such circumstances the toxic effects are of approximately equal severity in obstructed and nonobstructed lobules. When a difference in this regard exists, the nonobstructed lobules are damaged more severely than the obstructed ones.

In connection with this curious phenomenon, certain interesting possibilities suggest themselves. In a previous study of changes in the liver following ligation of single hepatic ducts [2b] we found evidence of active regeneration of hepatic cells in the obstructed lobules throughout periods of stasis of from one day to approximately five months. These changes were most evident in the inner portions of the lobules and consisted of hyperchromatism, binucleation, mitosis and the presence of atypical, flattened, newly regenerated cells. Mitotic figures were most numerous between the twelfth and thirty-fifth days. Lacquet [3] and Anderson [4] found that the recently restored liver (from

3. Lacquet, A. M.: Proc. Staff Meet., Mayo Clin. **5**:215, 1930.
4. Anderson, R. M.: Arch. Path. **14**:335, 1932.

two to four weeks after partial hepatectomy) was less susceptible than the normal liver to the hepatotoxic effects of carbon tetrachloride and chloroform. It may be that the same resistance to injury was exhibited by the actively regenerating and newly regenerated hepatic cells present in the obstructed lobules at the time of exposure to carbon tetrachloride in the present experiment (two weeks after ligation of hepatic ducts). However, the apparently diminished susceptibility of the nonobstructed lobules, which were not obviously significantly affected by ligation of single ducts, cannot be attributed to this phenomenon.

Love [1] found that following ligation of the common bile duct of the rat the hepatotoxic effect of phosphorus was observed sooner, but the lesion was not as extensive as, and disappeared more quickly than, that produced by a similar quantity of the drug in the previously normal liver. He suggested that this apparent resistance of the liver in biliary stasis might be due to the fact that ligation of the common bile duct interrupts the vicious circle of continued excretion of phosphorus in the bile and reabsorption of it from the intestine which occurs in the intact animal. Such a hypothesis cannot be invoked in the present case for two reasons: (1) The duct system draining usually more than half the liver was patent; (2) according to Wells,[5] about 96 per cent of absorbed carbon tetrachloride is eliminated by way of the lungs.

Goldschmidt, Vars and Ravdin [6] showed that the susceptibility of the liver to the toxic effect of chloroform varies more or less in direct proportion to its fat content as influenced by diet. In this connection it is interesting to note that we have found that, whereas in cats with total stasis of bile stainable lipid practically disappears from the liver after the seventh day,[2d] in those with single duct ligation there is abundance of this material in both obstructed and nonobstructed lobules after periods of stasis up to sixty-two days.[2b] It appears, therefore, that the apparent increase in resistance to carbon tetrachloride in our animals with obstructed bile ducts cannot be attributed to a decrease in the fat content of the liver, although no accurate quantitative chemical data are available. Nor does the possibility seem likely that this increase in resistance may be dependent on changes in hepatic circulation incident to the stasis of bile. Little or no significant morphologic evidence of such changes can be demonstrated with stasis in a single lobe of two weeks' duration. Moreover, the apparent resistance of the lobules without obstruction can scarcely be explained on any such basis.

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5. Wells, H. S.: Proc. Soc. Exper. Biol. & Med. **22**:235, 1935.

6. Goldschmidt, S.; Vars, H. M., and Ravdin, I. S.: Arch. Path. **25**:754, 1938.

The fact that there is usually no significant difference between the lesions in obstructed and nonobstructed lobules renders it unlikely that the increase in resistance to the poison is dependent on morphologic changes incident to stasis of bile. Forbes and Neale and their associates [7] found that there is present in aqueous extracts of liver a substance, apparently sodium xanthine, which protects the liver of the rat against the toxic effect of carbon tetrachloride. The ameliorating influence of ligation of single ducts may be due to the presence in the liver of an increased quantity of this protective substance. No data are available at present to support the foregoing hypothesis.

No satisfactory explanation is apparent for the secondary recrudescence of regressive phenomena in both groups of animals. Although it seems difficult to assume that absorption of the poison continues over a period of from two to three weeks, any other explanation for this phenomenon appears still less plausible.

SUMMARY

In adult cats the subcutaneous injection of carbon tetrachloride (0.3 cc. per kilogram) is followed by the development of rather characteristic regressive and regenerative phenomena in the central zones of the hepatic lobules.

In cats subjected to ligation of single hepatic ducts two weeks previous to the injection of carbon tetrachloride the hepatotoxic effect of this poison is distinctly less marked than in previously normal animals. This difference is strikingly evident in both the hepatic polygonal cells and the Kupffer cells.

Except at two and eight days following the injection, the regressive changes are of approximately equal severity in obstructed and nonobstructed lobules; at those stages the damage is distinctly more marked in the latter.

The morphologic differences between the two groups of animals are reflected in the functional activity. The abnormalities in bilirubinemia, retention of bromsulphalein and urobilinuria are less marked in those with ligation of single ducts than in the previously normal animals.

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AN ANEURYSM OF A CORONARY ARTERY

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An aneurysm of the coronary artery was discovered during a routine autopsy under Dr. L. D. Dhavale in a case of an aneurysm of the abdominal aorta. The specimen is chiefly described because of the comparative rarity of an indisputable coronary aneurysm.

Clinically, a large pulsatile tumor was noted in the epigastric region. The symptoms were pain in the back for three months, pain in both lower limbs and inability to walk. There was a definite history of a sore on the penis some years before, but the Kahn and Wassermann reactions on two successive occasions were negative. The clinical diagnosis of an aneurysm of the abdominal aorta was confirmed by roentgenologic examination. In the chest a rumbling sound, mainly systolic, was heard at the third left intercostal space. The patient suddenly collapsed, with a thready running pulse, and died. An antemortem diagnosis of ruptured aneurysm of the abdominal aorta was made.

At autopsy a very large retroperitoneal hematoma was found surrounding the two kidneys and the duodenum. In the center of the mass was a loculated aneurysm of the size of a fist, with smooth, thickened walls, projecting anteriorly. There was no visible rupture on the free surfaces. The aneurysm was not adherent to any retroperitoneal or intraperitoneal structures and had not eroded the vertebral column.

The aorta was of normal thickness and was not adherent to the surrounding structures. When it was opened, it showed a smooth surface with no atheroma or scarring. The opening of the celiac axis was changed into a transverse slit, and it was this artery rather than the aorta which had formed the aneurysm. Embedded in the wall of the aneurysm was the further course of the celiac branches above and of the superior mesenteric below. The rupture of the aneurysm was a longitudinal slit between the aorta and the aneurysm.

The ascending and thoracic portions of the aorta were free from gross evidence of arteriosclerosis or syphilis.

When the pericardium was opened, the left part of the coronary sulcus showed a thick-walled, irregularly oval bulging, 1.5 by 1 cm., in transverse direction. There were no other abnormalities in the heart. On dissection, the bulging proved to be an aneurysm of the left coronary artery, just beyond its commencement.

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When the heart was opened, the aorta and the sinuses of Valsalva were found to be normal. The opening of the left coronary artery was changed to a transverse slit into which a partly organized crumbling thrombus projected, but the lumen was not obliterated. Three millimeters beyond the aortic opening, the upper portion of the coronary artery had dilated into an oval aneurysm, 1.5 by 0.5 cm. in dimension. Beyond the aneurysm, the coronary artery felt more stiff than normal but showed no other gross abnormality. The aneurysm was filled with a laminated clot. The pericardium was thickened around the aneurysm into a dense fibrous covering. The right coronary artery was normal. The distal course of the left coronary artery was normal. The musculature of the heart showed no gross changes.

The Kahn and Wassermann tests with postmortem serum were positive. The liver showed characteristic gross scarring and lobulation.

Histologically, the aorta showed at places well marked medial degeneration, perivascular infiltrations and intimal thickening with diffuse mononuclear infiltration and formation of new capillaries.

The liver showed spreading fibrosis and mononuclear infiltration from the capsule into the substance.

The aneurysm of the coronary artery showed a normal aorta quite separate from the aneurysm. In the immmediate neighborhood of the aneurysm the media of the aorta was extensively disorganized by fibrosis, mononuclear infiltration and degeneration of musculoelastic tissue. The pericardium around the aneurysm was condensed into a dense fibrous tissue and showed many gummatous areas as well as diffuse mononuclear infiltration. A part of the wall of the aneurysm near the coronary artery showed a few persistent bands of muscle, and a portion of internal elastic lamina could be seen. Beyond this the whole of the wall was formed by fibrous tissue showing hyaline degeneration at places, dense near the lumen and loose toward the pericardium. The neighboring coronary branches showed well marked intimal thickening. Gram's stain showed no organisms. No spirochetes could be detected by silver staining.

Authentic aneurysms of the coronary artery are rare. Packard and Wechsler [1] could collect 31 previous records but concluded that two thirds of the specimens recorded were aneurysms of sinuses of Valsalva. Packard described one more specimen. In this department, in 2,300 postmortem examinations, aneurysms of the sinuses of Valsalva were met with in 4 autopsies, and the present specimen is the only one derived from the coronary artery. Of the 4 aneurysms of the sinuses of Valsalva, 2 had their origin in emboli due to infection, one presum-

1. Packard, M., and Wechsler, H. F.: Arch. Int. Med. **43**:1, 1929.

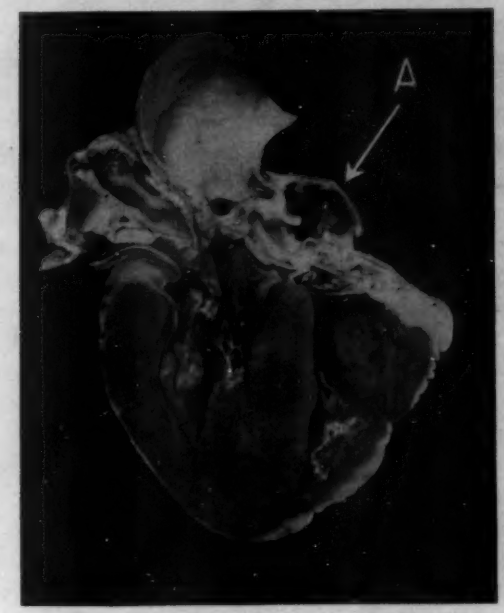

Fig. 1.—Gross appearance of the aneurysm (*A*).

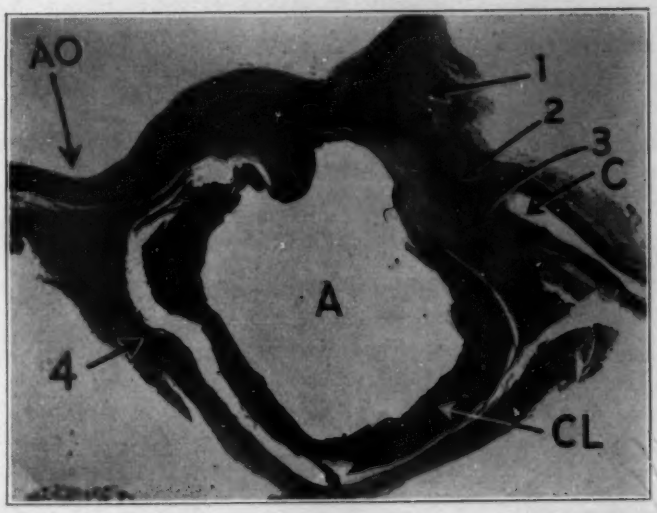

Fig. 2.—Histologic appearance of the aneurysm (*A*); × 3. *Ao* designates the aorta; *C*, the coronary artery; *Cl*, the blood clot; *1* to *4* gummas in the wall of the aneurysm.

ably tuberculous and one syphilitic. Schuster [2] described an aneurysm, but the coronary artery was simply incorporated in its wall. Cox and Christie [3] described an aneurysm on the right coronary artery. The aneurysm was considered to be arteriosclerotic in origin, but the person also had an aneurysm of the abdominal aorta, which ruptured with fatal results. Nagayo and Takahashi,[4] Harris [5] and others have described multiple dilatations on the coronary arteries. Nagayo and Takahashi named this condition serpentine aneurysm. Such aneurysms are described in animals as well. Packard and Wechsler, reviewing the symptomatology, found that 50 per cent of the aneurysms were incidental but that in the remaining 50 per cent the signs and symptoms were bizarre. In a case described by Eliasoph,[6] thrombosis from the aneurysm spreading into the coronary vessel itself had caused an aneurysm of the left ventricle and interventricular septum. Rae [7] described an aneurysm on each coronary artery.

Regarding the causes, males were found to be more commonly affected than females. No age group was specially liable. Packard and Wechsler appear to be inclined to agree with recent observers in minimizing the role of syphilis in the causation of aneurysms of smaller vessels. The majority of aneurysms have been of the mycotic embolic type, the emboli being derived from vegetations on the aortic valves. Rheumatic infection was present in 17 cases. Arteriosclerosis was the next most frequently observed cause, and here the possibility of solitary arteriosclerosis of the coronary vessels at an early age is emphasized. In only 3 cases was the aneurysm definitely syphilitic. Subsequent to the publication of the review by Packard and Wechsler, Seydel [8] described a syphilitic aneurysm on the left descending coronary branch. Snyder and Hunter [9] also described a case, but in this the sinus of Valsalva was first involved and the coronary artery was secondarily invaded.

The present specimen appears to be of syphilitic origin, closely resembling in histologic appearance those described by Moritz.[10]

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3. Cox, R. L., and Christie, C. D.: Am. J. M. Sc. **180**:37, 1930.
4. Nagayo, M., and Takahashi, H.: Tr. Jap. Path. Soc. **22**:583, 1932.
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10. Moritz, A. R.: Arch. Path. **11**:44, 1931.

POSTMORTEM BLOOD CHEMICAL DETERMINATIONS

A COMPARISON OF CHEMICAL ANALYSES OF BLOOD OBTAINED POST MORTEM WITH DEGREES OF RENAL DAMAGE FOUND AT AUTOPSY

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For many years clinical investigators have attempted to correlate the results of various clinical tests of renal function with the pathologic changes found at autopsy. The recognition of a wide discrepancy between the clinical and the anatomic facts has resulted in the development of a great number of laboratory procedures for the study of various substances in the blood and urine in the hope of determining the pathologic physiology of the kidney. Such prominent clinical investigators as Christian [1] and Schlayer [2] have indicated their belief that the changes in renal function are in themselves independent of the type of anatomic disease, that there is no close relationship between diffuse diseases and functional changes in the kidney and that similar anatomic lesions may cause entirely different functional changes.

The failure of these various tests of renal function to reveal the nature of the lesion in the kidney has resulted in more intensive efforts to interpret clinical findings on the basis of known structural changes. Volhard and Fahr [3] in 1914 and Addis and Oliver [4] in 1931 were able to group their cases clinically and predict the type of renal lesion later found at autopsy. While certain discrepancies were noted, sufficient progress was made to indicate that it is necessary to return to Bright's original precept that disease must be explained by the study of morbid anatomy.

The pathologist, too, is frequently confronted with the perplexing task of correlating the structural changes found at autopsy with limited clinical and laboratory data. The presence of one or more disease processes in other organs, any one of which might be sufficient to cause death, increases the difficulty experienced in determining the role

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From the laboratories of the St. Francis and Passavant Hospitals.

1. Christian, H.: Tr. A. Am. Physicians **44**:68, 1929.

2. Schlayer, K. R.: Beihefte z. med. Klin. **8**:211, 1912.

3. Volhard, F., and Fahr, T.: Die Brightsche Nierenkrankheit, Berlin, Julius Springer, 1914.

4. Addis, T., and Oliver, J.: The Renal Lesion in Bright's Disease, New York, Paul B. Hoeber Inc., 1931.

played by the lesion found in the kidney. The estimation and interpretation of gross and microscopic changes in the kidney must always be made with the realization that during the last few hours of life, and even more rapidly after death, bacterial invasion, tissue disintegration, chemical changes and various other autolytic phenomena may alter the final picture. The type of disease process, the temperature of the body after death, and the interval of time between death and autopsy are other factors which also influence chemical decomposition. In an effort to avoid these postmortem changes the pathologist obtains cultures, fluids and tissues for chemical and microscopic study as soon after death as possible.

The possibility of some correlation between the chemical composition of blood obtained post mortem and the structural changes found in the kidney at autopsy prompted the study of a series of chemical analyses of blood obtained in 104 autopsies. The study included determinations of blood sugar, nonprotein nitrogen, creatinine and chlorides. The conditions disclosed in the autopsies were classified as acute infections, miscellaneous diseases, intestinal stasis and Bright's disease. The study of the kidneys included gross and microscopic examination. The latter was made on a number of microscopic sections from each kidney, so that a fairly complete composite picture of the renal architecture was obtained. The renal lesions were at first divided into negative, very slight, slight, moderate and severe; but later the first three divisions were grouped as slight since it was believed that such structural changes would be unable in themselves to affect the results of a chemical study of the blood.

No attempt was made to compare all the chemical determinations on antemortem and postmortem blood, because antemortem tests were made in only 44 cases of the entire series, and in many of these there was a lapse of a considerable number of days between the test and the death of the patient. Suffice it to say that generally the results obtained before and after death agreed only in those cases in which a clinical diagnosis of Bright's disease was made and later confirmed at autopsy.

TECHNIC

The chemical determinations were made on serum obtained by separation of blood removed from the auricle. Serum was used because of the presence of clots in postmortem blood. The concentrations of sugar, nonprotein nitrogen and creatinine in serum and whole blood are essentially the same, but the chlorides are higher in serum than in whole blood.

After the heart was exposed, the pericardial sac and heart were mopped dry with a towel to prevent contamination of the auricular blood with other body fluids. The heart was lifted up by its apex, and a clean dry finger bowl or wide-mouthed bottle was slipped under the right auricle. Then with scissors or knife a longitudinal slit was made in the lateral wall of the heart. The blood

was allowed to flow or was milked into the receptacle, care being taken to contaminate the blood as little as possible. It was necessary to remove from 25 to 50 cc. of blood, since the presence of clots frequently reduced the expected yield of serum. Some of the technical difficulties encountered included the interposition of voluminous or consolidated lungs, an autopsy technic altered because of an unusual pulmonary or cardiac lesion or a firmly adherent blood clot in the auricle which prevented the escape of fluid blood. As a result the autopsies concerned here were not consecutive. An insufficient amount or marked hemolysis of the serum prevented analysis in a few cases.

The blood was obtained within from one to twenty-four hours after death, and chemical analysis was usually made within two hours. The analysis for blood sugar was done according to the method devised by Folin and Wu; the nonprotein nitrogen and creatinine were determined as by Folin and the chlorides by the Whitehorn modification of the Volhard method.

Summary of Observations

Classification of Cases	Cases	Postmortem Creatinine Values, Mg. per 100 Cc. of Blood	Estimate of Renal Damage: Slight — Cases	Slight — Postmortem Creatinine Values	Moderate — Cases	Moderate — Postmortem Creatinine Values	Severe — Cases	Severe — Postmortem Creatinine Values
Total series	104	.....	65	1.27-7.60, Av. 2.46*	21	1.56-8.12, Av. 3.36	18	1.94-11.5, Av. 5.03
1. Acute infections	25	1.32-4.29, Av. 2.76	19	1.32-4.21, Av. 2.67	5	2.14-4.27, Av. 3.12	1	2.61
2. Miscellaneous diseases	56	1.46-6.37, Av. 2.77	39	1.46-3.85, Av. 2.25	10	1.56-4.69, Av. 3.08	7	4.29-6.37, Av. 5.20
3. Intestinal stasis	13	1.69-8.12, Av. 3.58	7	1.69-7.60, Av. 3.20	5	1.96-8.12, Av. 4.38	1	2.16
4. Bright's disease	10	1.94-11.50, Av. 4.63	..	.....	1	2.22	9	1.94-11.50, Av. 4.90

* Av. denotes average.

The changes in volume of blood resulting from evaporation, dehydration and diffusion during the last few hours of life and after death and the variation in individual stability of the various constituents of the blood are some of the factors which probably affected the values obtained.

ANALYSIS OF BLOOD CHEMICAL DETERMINATIONS

Sugar.—The variations in the postmortem determinations of blood sugar were extreme and suggested that this is probably the most labile of the blood constituents examined. In 50 cases the determinations were below 10 mg. per hundred cubic centimeters of blood. The highest was 646 mg., on blood from a man with acute lobar pneumonia. In this instance there was no history of diabetes; no chemical examination of the blood had been made during life; the urine was sugar free on admission, and the giving of a moderate amount of dextrose intravenously as a therapeutic measure had been discontinued six hours

before death. Of the 16 persons whose blood sugar was above 150 mg., 7 had died of some cerebral involvement likely to produce intracranial pressure; there were 2 with brain tumors, 2 with cerebral hemorrhage and 3 with meningitis. Possibly the high levels of blood sugar were due to the increased intracranial pressure on the "sugar center" in the medulla. However, in other cases in which the pathologic changes in the brain were similar, the postmortem blood sugar determinations were normal or very low.

There were 2 patients with diabetes mellitus in the entire series. One died in diabetic coma, but both the final antemortem and the postmortem value for the blood sugar were within normal limits. In the other diabetic person's blood the sugar two days before death was 180 mg., while post mortem it was less than 10 mg.

The frequency of low values for agonal and postmortem blood sugar has been reported by other writers. Both Paul [5] and Schmidt [6] suggested that hypoglycemic states may be a common complicating factor in the terminal or agonal stages of a variety of conditions. Mann demonstrated that complete removal of the liver causes a rapid fall in blood sugar in dogs. In severe hepatic damage caused by cancer or acute yellow atrophy low values for blood sugar are frequently obtained during life. In this and in other similar studies, no evidence of damage to the liver was found sufficient to account for the hypoglycemia.

Balakhovskiy and Ginzburg [7] studied the chemical changes in postmortem blood preserved for transfusions and found the blood sugar high in 13 of 28 cases. The chemical analyses were often made from four to six days after death, and they assumed that since glycolytic processes had probably occurred the blood sugar had been even higher when the blood was collected. They found that the blood removed from the hearts of normal dogs several hours after the animals were killed invariably showed high blood sugar, while that of dogs whose livers had been removed before the killing of the animals disclosed no change in the level of blood sugar. They concluded that the liver was the source of the sugar causing the increase in the postmortem blood sugar determinations. They also observed that the rate of glycolysis in preserved blood, with subsequent reduction in the values for blood sugar, varied greatly. They assumed that a disintegration of glycoproteids, nucleoproteins or other sugar combinations may release reducing substances and cause the occasional rise in values which they observed.

Postmortem glycolysis does not satisfactorily explain the great variation in the values obtained in this series. Possibly an increase or

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6. Schmidt, E. G.: Arch. Int. Med. **47**:128, 1931.

7. Balakhovskiy, S. D.; Ginzburg, F. G., and others: Biochem. Ztschr. **252**: 370, 1932.

a decrease in hepatic glycogenolysis or in tissue utilization of dextrose due to an alteration in metabolism during the terminal or agonal stages of a number of conditions may result in high or low postmortem blood sugar determinations.

Nonprotein Nitrogen.—The nonprotein nitrogen determinations varied from 32 to 342 mg. per hundred cubic centimeters of blood, and over half of the readings were above 100 mg. In the presence of Bright's disease the nonprotein nitrogen was always high, but high readings were also obtained in cases in which no renal damage could be demonstrated at autopsy.

Sander [8] found that the nonprotein nitrogen increased rapidly and continuously. Paul [5] noted that the degree of increase was irregular and was more pronounced in those cases in which there was a high figure at the start. The average represented a 30 per cent increase in twenty-four hours. Repeated determinations made on a number of the blood samples in this series showed a steady and sometimes marked rise in nonprotein nitrogen.

Chlorides.—The chloride content of blood serum obtained post mortem fluctuated widely, ranging from 250 to 722 mg. per hundred cubic centimeters of blood. Grouping of the various diseases likely to cause either hyperchloremia or hypochloremia showed no relationship to the chloride determinations. All investigators have agreed that the fluctuations in the chlorides of blood obtained post mortem are marked and irregular and their study of little value.

Creatinine.—Creatinine is a product entirely of endogenous metabolism, and its formation and elimination are normally very constant. Myers and Lough [9] were the first to show that a rise in blood creatinine is a more important sign of loss of renal function than a rise in either uric acid or urea, which are both exogenous in origin. They stated that a creatinine determination above 5 mg. indicates a fatal outcome unless the retention is due to some acute renal condition.

In a study on the preservation of blood for chemical analysis Sander [8] found that the creatinine values of unpreserved blood remained constant for six days. This remarkable stability of creatinine had been noted previously by Falk, Baumann, and McGuire.[10] Repeated determinations on the same blood samples, secured from several autopsies in this series, showed little change in the creatinine values under a variety of conditions.

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8. Sander, F. V.: J. Biol. Chem. **108**:1, 1923.

9. Myers, V. C., and Lough, W. G.: Arch. Int. Med. **16**:536, 1915.

10. Falk, K. G.; Baumann, E. J., and McGuire, G.: J. Biol. Chem. **37**:525, 1919.

Polayes, Hershey and Lederer [11] compared the creatinine and urea contents of antemortem and postmortem blood in 100 cases. They found that the values for creatinine were more helpful than those for urea in determining the state of renal function during life. They concluded that a creatinine content of 4 mg. or more per hundred cubic centimeters of blood obtained post mortem indicated severe renal insufficiency during life.

COMMENT ON GROUPS

Since the creatinine of blood obtained post mortem appeared to be the only stable constituent examined, the values for this constituent were selected for comparison with the degrees of renal damage found at autopsy.

Acute Infections.—This group of 25 cases included 16 cases of acute lobar pneumonia, 3 of pneumococcic meningitis and 3 of streptococcic septicemia. In 19 cases in which renal damage was slight or absent the creatinine readings ranged from 1.32 to 4.21 mg. The highest reading of 4.21 mg. was made on the blood of a tabetic patient in whom severe streptococcic cellulitis and septicemia developed following an abrasion of the finger. The renal damage was slight. In the 5 cases in this group in which renal damage was moderate and the one in which it was severe the patients all died of acute lobar pneumonia. None of the clinical findings suggested a diagnosis of Bright's disease, and yet the postmortem creatinine content ranged from 2.14 to 4.29 mg.

Miscellaneous Group.—In this group of 56 cases there were 10 of malignant tumor, 5 of chronic heart disease, 3 of sudden accidental death, 3 of delirium tremens, 2 of hyperthyroidism and 1 each of Banti's disease, Addison's disease, portal cirrhosis, tuberculous meningitis and bronchial asthma. The renal damage was estimated as slight in 39 cases, moderate in 10 and severe in 7 cases.

The highest reading of 6.37 mg. was made on the blood of a woman 81 years of age with marked arteriosclerotic nephritis and primary cancer of the liver. Of the 17 cases in which renal damage was moderate or severe, 4 were cases of chronic heart disease with cardiac failure. The circulatory failure through a kidney already damaged explains the retention of creatinine. In 8 cases in this group the creatinine reading was above 4 mg. In 5 of these a chemical study of the blood had been made from three to twelve days before death; in only a single case was the creatinine reading above 2 mg.

In all of the 7 miscellaneous cases in which renal damage was severe the postmortem blood creatinine was above 4 mg. Of these cases, 4 were of chronic heart disease, 2 of malignant tumor and the

11. Polayes, S. H.; Hershey, E., and Lederer, M.: Arch. Int. Med. **46**:283, 1930.

seventh was a case of cerebrospinal syphilis. The severe renal lesion in each of these cases was not revealed by the ordinary clinical and laboratory findings. During the terminal stages renal insufficiency probably played an important and unsuspected role in the fatal outcome.

Intestinal Stasis.—For want of a better term, "intestinal stasis" is used to include 13 cases of intestinal obstruction, peritonitis and paralytic ileus. In 6 of these cases generalized peritonitis had resulted from rupture of an appendix, a duodenal ulcer or a cancer of the large intestine. There were 4 cases of intestinal obstruction and 2 of a paralytic ileus.

The highest creatinine reading of 8.12 mg. was made on the blood of a 64 year old woman with arteriosclerotic thrombosis of the superior mesenteric artery, causing gangrene of almost the entire small intestine. The arteriosclerotic nephritis was of moderate severity. The high reading of 7.26 mg. was obtained in the case of a woman aged 27 years with only slight renal lesions. Chemical examination of blood taken the day before death showed nonprotein nitrogen 29.2 mg. and creatinine 1.27 mg. At autopsy a cancer of the sigmoid colon with intestinal obstruction and generalized peritonitis was found.

Bright's Disease.—In the entire series there were 10 clinical cases of Bright's disease, and at autopsy in each case this diagnosis was confirmed as the immediate cause of death. All of the patients showed renal damage, moderate or severe. Of the 4 with postmortem creatinine values below 4 mg., 3 had severe renal damage and died of uremic coma, clinically diagnosed. In the 6 patients with creatinine determinations above 4 mg. the renal lesions were estimated as severe. In the latter group there were 3 on whose blood chemical analyses were made ante mortem and all of them had creatinine readings above 4 mg. Death of the other patients within a few hours after admission to the hospital prevented antemortem chemical studies of the blood.

Comparison of the figures for postmortem creatinine with the estimations of renal damage in the entire series without regard to the primary cause of death showed several interesting findings: There were 65 cases in which renal lesions were slight or absent. In these instances the average creatinine figure was 2.46 mg. In 2 cases in which renal damage was slight the postmortem creatinine determination was above 4 mg., and in 10 cases the reading was above 3 mg. There were 21 cases in which renal damage was moderate, and the average figure for postmortem creatinine was 3.36 mg. In 6 cases the creatinine reading was above 4 mg., and in 13 cases it was above 3 mg. In the group of 18 cases in which renal damage was severe, there were 12 cases in which the postmortem creatinine was above 4 mg. The average creatinine

figure for this group was 5.03 mg. In 9 cases, or one half of this group, the clinical diagnosis was Bright's disease, which was later confirmed at autopsy. Moderate or severe renal damage was found in 10 cases in which the creatinine determination on blood obtained post mortem was within normal limits. In 45 cases there was slight or no demonstrable renal damage although the creatinine in the postmortem blood was above normal.

SUMMARY

Determinations of the sugar, nonprotein nitrogen and chlorides in blood obtained post mortem are of no value in the correlation of clinical facts with structural changes found at autopsy. The values for these chemical constituents vary greatly. The values for postmortem blood sugar quoted by other writers are at opposite extremes, and the theories advanced to explain these variations from the normal seem inadequate. Variations in hepatic glycogenolysis and in tissue utilization of dextrose due to an alteration in metabolism during the agonal stages of disease offer a more likely explanation of the fluctuating sugar values.

Of all the chemical constituents of the postmortem blood, creatinine is the most stable. At first glance the average figures given for creatinine in the several groups in this series seem to indicate that the variations in creatinine in postmortem blood are specific and related to the structural changes in the kidney. However, the many exceptions in each group independent of the amount of renal damage present show that the value for creatinine in blood obtained post mortem cannot be taken as an index of renal function at the time of death. The finding of a very high level of creatinine in postmortem blood in some cases in which there was little or no demonstrable damage of the kidneys suggests that the retention of this particular product of metabolism under certain conditions may depend on factors other than structural changes in the kidneys. A failure of circulation through the renal vascular bed and toxemia are the most likely factors which may cause a retention of creatinine during the agonal period. Of special interest was the frequency with which severe renal damage and a postmortem creatinine value below 4 mg. were found in the same case. There were 5 such cases in the entire series. In 3 of these the diagnosis was Bright's disease with uremia, and in 2 of the 3 cases the postmortem creatinine reading was within normal limits. In these cases one must conclude that one type of renal function is retained, and this may explain why certain patients with severe renal damage live so long.

CONCLUSIONS

The concentrations of sugar, nonprotein nitrogen and chlorides in blood obtained post mortem vary greatly, and their determination is of little value.

Creatinine is the only stable chemical constituent of postmortem blood.

The creatinine content of blood obtained post mortem is no indication of the amount of renal damage present.

A postmortem creatinine determination cannot be regarded as an index of renal function during life.

Chemical analysis of blood obtained post mortem is recommended because of its value in a few cases.

EFFECT OF VITAMIN D_2 (CALCIFEROL) ON THE DOG

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A survey of the literature of the biologic and pathologic effects of vitamin D has brought us to the conclusion that no further progress can be made unless the pathologist and the pharmacologist work in close collaboration. It is in this spirit that the work reported here was begun and achieved. For reasons which will become apparent the dog is more suitable than the rabbit for work of this kind. References to the rabbit will be made only occasionally. By comparing systematically dosage and physiologic and histologic changes we have gained a clearer insight into the pharmacologic aspects of vitamin D and have found a new experimental approach to the study of arterioles and of renal diseases.

MATERIAL AND METHODS

We have given by mouth a solution of pure calciferol in varying concentrations in arachis oil to young adult dogs. These animals, judged by the condition of their teeth, were not more than 3 years old. Males were used in preference to bitches, which were taken under observation only occasionally to complete our series. Our experiments have extended over a period of three years. Yet in some respects we consider ourselves only at the end of a period of orientation, during which we have gathered enough information to enable us to extend our investigations on a more choice material. The diet, though not constant, varied little qualitatively and quantitatively and included a fair proportion of meat. In winter the animals were kept in a room at uniform temperature. They were killed by exsanguination. Complete gross examination rarely disclosed macroscopic changes (e. g., gastric hemorrhage) except when remarkable lethal doses were given.

Slices of organs were fixed, except the heart, intestines and brain, which were kept in a mixture of 40 per cent solution of formaldehyde and physiologic solution of sodium chloride (9:1). A short fixation in Zenker-formaldehyde solution (twelve hours) followed by a twenty-four hour immersion in potassium bichromate 3 per cent gave the best results for the study of the arterioles. With the Bouin-

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Hollande fixation, the myofibrils stand out clearly. Several staining methods, including the orcein stain, were applied, but the largest use was made of Masson's trichrome methods, which are indispensable for a thorough study of the arteriolar wall. Mallory's phosphotungstic acid-hematoxylin was also useful for an analysis of refined histologic structures; calcium deposits were determined by the Kossa method in conjunction with hematoxylin staining.

The detailed nature of our investigations and the small size of the smooth muscle cells have obliged us to resort to camera lucida drawings. Of suitable preparations, photomicrographs were made (Mr. Pittock).

Our material is tabulated according to the dosage, duration of treatment and mode of administration of the solution of calciferol (tables 1, 2 and 3). These tables give a summarized account of the changes in the blood pressure, blood calcium, renal arterioles and parenchyma.

The data on four adult dogs (Chris., Ben., Ger. and Alf.) given respectively a single lethal dose of 13, 15.8, 16.7 and 20 mg. per kilogram, and five dogs (Pros., Clem., Louis, Gab. and Leon) given respectively a single dose of 2.9, 4, 6, ±7 and 8 mg. per kilogram are not tabulated.

THE ARTERIOLES OF THE NORMAL KIDNEY

We have centered our observations on the arterioles of the kidney. Arteriolar changes in the spleen, pancreas and adrenal are discussed where indicated.

It may seem unnecessary to devote a special section to the histology of normal renal arterioles. Yet the classic descriptions are inadequate for the task with which we are confronted. We give here the results of our recent investigations on the subject, summarized in a recent paper (Goormaghtigh [1]).

Heterogeneous Structure of the Media.—The media of the renal arterioles has a heterogeneous structure; it contains two different types of cells: the ordinary smooth muscle cell and an afibrillar or paucifibrillar cell (fig. 1 *A, B, C* and *D;* fig. 2 *A* and *B*).

In 1932, one of us (Goormaghtigh [2]) drew attention to a group of afibrillar cells in the arteriolar wall of the vas afferens, close to the glomerulus, which he named the "juxta glomerular neuro-myoarterial segment or apparatus" (fig. 2 *A*). Ruyter [3] in 1926 discovered this structure in the mouse and Oberling [4] extended the observations to the human kidney. Unaware of our publication, Zimmermann [5] coined for this finding the word *Polkissen.*

The afibrillar cells (afibrillar myoblasts) are considered as specially differentiated leiomyoblasts (Ruyter; Oberling; Goormaghtigh; Zimmermann). Ruyter and Goormaghtigh described transitional types of

1. Goormaghtigh, N.: J. Physiol. **90**:1263, 1937.
2. Goormaghtigh, N.: Arch. de biol., Paris **43**:575, 1932.
3. Ruyter, J. H. C.: Ztschr. f. Zellforsch. u. mikr. Anat. **2**:242, 1925.
4. Oberling, C.: Compt. rend. Acad. d. sc. **184**:1200, 1927.
5. Zimmermann, K. W.: Ztschr. f. mikr.-anat. Forsch. **32**:176, 1933.

cells, provided with a few myofibrils (paucifibrillar cells or myoblasts). Ruyter and Schumacher considered the afibrillar elements as epithelioid cells; the paucifibrillar myoblasts have a spindle shape. The large nucleus is spherical, ellipsoid or irregular. Binuclear cells are sometimes observed. After Regaud's fixation, the protoplasm is clearer than that of the contractile elements. Ruyter and Goormaghtigh demonstrated mitochondrial granules in the protoplasm (mouse, cat).

We wish to emphasize the fact that the afibrillar cells are not confined to the juxtaglomerular segment. They are found in the interlobular prearteriole of the kidney, chiefly under the internal elastic

TABLE 1.—*Continuous Treatment*

Dog	Vitamin D, Micrograms per Kg. Daily	Duration of Treatment, in Days	Blood Pressure, Mm. of Hg		Serum Calcium, Mg. per 100 Cc.		Minimal Active Dose of Epinephrine, Micrograms per Kg.	Effect in Renal Arterioles
			Before Treatment	After Treatment	Before Treatment	After Treatment		
1	60	10	130	126	9.8	9.8	.....	No change
2	70	37	...	...	10.5	11.0	.....	
3	60	95	130	126	9.3	9.8	Reaction Inverted	Incipient hypertrophy
4	50	95	132	144	10.6	12.2	Reaction Inverted	Incipient hypertrophy
21	50	102	135	166	10.2	11.2	Reaction Inverted	Hypertrophy
22	40	102	138	165	9.8	13.0	Reaction Inverted	Hypertrophy
10	160	8	136	136	9.8	11.6	0.0005	Hypertrophy
6	180	6	128	142	9.6	10.2	.....	Hypertrophy
17	170	71	...	...	...	....	.....	
11	220	6	132	126	9.8	10.2	0.0005	Hypertrophy
7	230	10	135	172	9.4	10.5	0.0200	Hypertrophy
8	230	10	128	188	8.9	10.8	0.0050	Hypertrophy (especially of afibrillar cells)
14	230	24	136	172	8.4	9.8	0.0080	
15	230	25	132	170	8.2	10.0	0.0005	Hypertrophy (especially of afibrillar cells)
19	110	114	138	200	9.1	12.2	0.1000	Marked hypertrophy of both cell types
12	720	3	...	156	9.6	10.2	0.0400	Marked hypertrophy
13	720	5	...	178	9.6	9.4	0.0050	Marked hypertrophy

membrane. Their large nuclei stand out conspicuously (fig. 1 *D*). Around the origin of the vas afferens, the afibrillar cells form a differentiated ring, connected with the proximal and distal monocellular longitudinal subintimal bundle.

In the vas afferens the afibrillar leiomyoblasts form the main bulk of the juxtaglomerular apparatus (fig. 2 *A*) but are located also along the wall, either under the endothelium or on the outer surface, as already noticed by Ruyter and Oberling (fig. 1 *A, B* and *C;* fig. 2 *A* and *B*). A minute longitudinal bundle in connection with the neuromyoarterial apparatus is a constant feature.

In the efferent glomerular arterioles the afibrillar elements form a contrast with the small-sized smooth muscle cells and lie close to the places of bifurcation and capillarization.

TABLE 2.—*Continuous Treatment*

Dog	Vitamin D, Micrograms per Kg. Daily	Duration of Treatment, Days	Blood Pressure, Mm. of Hg		Blood Calcium, Mg. per 100 Cc.		Renal Arterioles*							(a) Calcium Excretion (b) Tubular Lesions	Glomerular Collapse	Interstitial Infiltration
			Before Treatment	After Treatment	Before Treatment	After Treatment		H	V	A	T	S	N			
20	520	14	128	185	9.7	12.8	N.M.A.	+++								
							a.l.l.	+	..	+	..	RRR	R	(a) 0	0	0
							a.gl.	++						(b) 0		
25	550	17	134	172	8.1	9.8	N.M.A.	+++								
							a.l.l.	+	+++	+	++	++	+	(a) 0	0	0
							a.gl.	+	+	+	R	+		(b) 0		
23	600	45	136	206	10.4	12.8	N.M.A.	++	+	..	..	..	..	(a) RRR	0	0
							a.l.l.	R	++	+	++	+++	++	(papilla)		
							a.gl.	R	+	+	+	++	+	(b) 0		
24	600	45	131	208	8.8	11.8	N.M.A.	++	+	..	..	..	..	(a) RRR	0	0
							a.l.l.	R	+++	+	++	+++	+++	(papilla)		
							a.gl.	R	0	+	+	++	++	(b) 0		
Bas.	600†	32	128	189	9.2	11.8	N.M.A.	0								
							a.l.l.	0	++	R	R	+	+++	(a) (papilla)		
							a.gl.	0	0	R	0	+	++	(b) R	0	0

* H signifies hypertrophy; V, vaculoation; A, atrophy; T, clear tumefaction; S, shinkage; N, necrosis; N.M.A., neuromyoarterial and afibrillar cells; a.l.l., interlobular arteriole a.gl., glomerular arterioles; R, rare or scarce; RRR, exceedingly rare.

† This dog received an initial dose of 3 mg. per kilogram and survived one hundred and fifty days.

TABLE 3.—*Intermittent Treatment*

Dog	Doses and Size of Dose, Mg. per Kg.	Interval Between Doses, Days	Duration of Treatment, Days	Survival After Treatment, Days	Weight, Kg.		Serum Calcium, Mg. per 100 Cc.		Renal Arterioles*							Calcium Excretion; Tubular Lesions	Glomerular Collapse	Interstitial Changes
					Before Treatment	After Treatment	Before Treatment	After Treatment		H	V	A	T	S	N			
Guil.	5×2.5 mg.	1	10	1	10.7	9.8	8.8	18.6	N.M.A.	+	0	0	0	0	0	++	++	0
									a.l.l.	+	+	+	0	0	0			
									a.gl.	+	0	0	0	0	0			
Phil.	2×5.0 mg.	4	10	1	10.1	8.3	8.7	15.3	N.M.A.	+	0	0	0	0	0	+++	++	+
									a.l.l.	0	++	++	++	+	R			
									a.gl.	0	+	++	R	R	RR			
Mad.	8×2.6 mg.	2	22	+	11.8	9.0	10.3	22.8	N.M.A.	+	0	0	0	..	..	+++	++	+
									a.l.l.	0	R	+++	++	++	+			
									a.gl.	0	0	++	R	+	R			
Ad.	9×2.0 mg.	4	45	2	8.3	5.5	9.1	16.5	N.M.A.	R	0	0	0	0	..	+++	+++	++
									a.l.l.	0	R	+++	R	++	+			
									a.gl.	0	0	++	0	+	R			
Herm.	7×1.5 mg.	6	51	2	6.2	3.0	10.1	23.5	N.M.A.	+	0	0	0	0	0	+++	+++	+++
									a.l.l.	0	R	+++	0	0	0			
									a.gl.	R	R	++	0	0	0			
Yol.	8×2.1 mg.	2	22	56†	19.8	16.9	10.1	23.6	N.M.A.	0	0	0	0	0	0	+	+	+
									a.l.l.	0	0	0	0	R	0			
									a.gl.	0	0	0	R	0	0			
Jean	11×2.6 mg.	3	41	48†	9.5	7.0	9.3	22.6	N.M.A.	RR	R	R	0	..	..	++++	++++	++++
									a.l.l.	0	0	++	0	+	R			
									a.gl.	0	0	+	0	+	R#			
Char.	11×3.6 mg.	3	41	28†	5.5	3.7	9.8	23.5	N.M.A.	0	R	0	0	0	0	++++	++++	++++
									a.l.l.	0	+	+	0	0	+			
									a.gl.	0	+	+	0	0	0			
Thér.	9×3.0 mg.	2	25	412	4.5	2.8	9.3	23.5	N.M.A.	0	0	0	..	..	..	+	+	++ (scar tissue)
									a.l.l.	0	0	0	..	RR	+			
									a.gl.	+§	0	0	..	RR	+			

* The abbreviations are explained under table 2.
† The dog died.
# Necrosis was rare except at bifurcations.
§ Myofibrils were scarce.

So far, the notion of these especially differentiated leiomyoblasts rests on a histologic basis. Also their mode of response to pathologic conditions distinguishes them from the ordinary smooth muscle cells.[6]

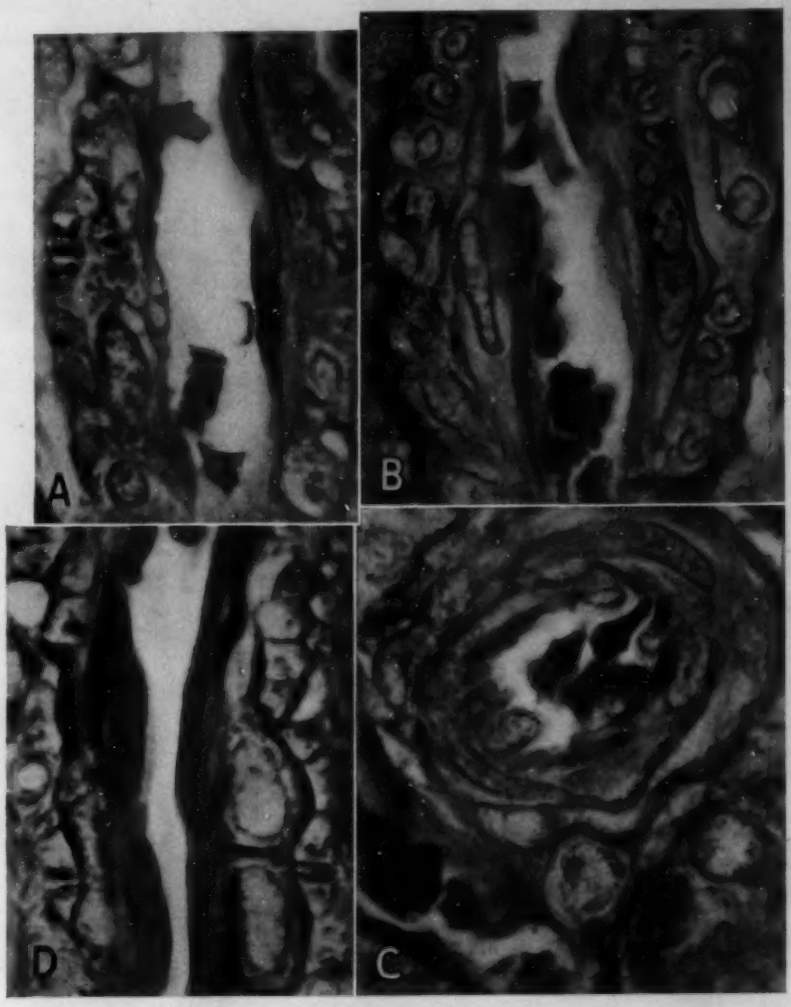

Fig. 1.—*A*, vas afferens in a young puppy, showing subendothelial clear afibrillar cell with three nuclei, contrasting with the dark outer layer composed of smooth muscle cells. *B*, corresponding vas afferens in a normal adult dog, showing subendothelial afibrillar cells. *C*, clear afibrillar cell lying on the surface of the vas afferens (normal dog). *D*, two large subendothelial afibrillar cells in an interlobular prearteriole (Kulschitsky stain); × 1,600.

The toxemia of acute scarlet fever differentiates the two types of cells in the small arterioles. The structural duality of the arteriolar media

6. Goormaghtigh, N.: Compt. rend. Soc. de biol. **124**:293, 1937.

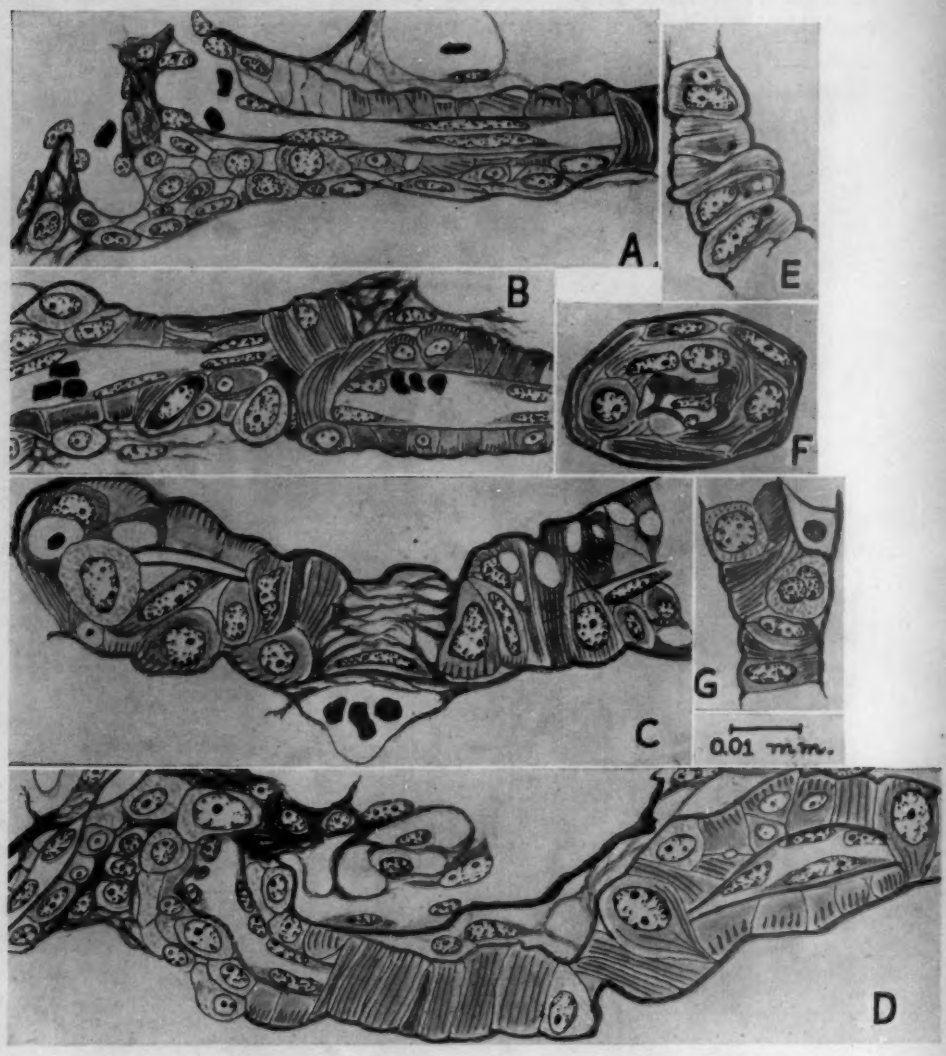

Fig. 2.—*A*, vas afferens of a normal dog (outer layer of the cortex), showing juxtaglomerular afibrillar cells (neuromyoarterial apparatus) connected with a peripheral bundle of afibrillar cells. *B*, vas afferens of a normal dog, showing a large afibrillar cell near the origin of a Ludwig arteriole. *C* and *D*, corresponding vasa afferentia of dogs 13 and 19, showing hypertrophy of the smooth muscle cells and afibrillar cells. *E*, tangential section of vas afferens of dog 8, showing fuchsinophil bodies and vacuolation. *F*, binuclear smooth muscle cell. *G*, binuclear afibrillar cell, probably exemplifying amitosis.

is thus confirmed by observations of morbid conditions. Our work on vitamin D affords further proof of this assertion. Our investigations demonstrate the systemic distribution of the afibrillar leiomyoblasts and their tendency to form small bundles in the neighborhood of arteriolar bifurcations or to accumulate at the sites of capillarization.

What is the physiologic significance of these cells? They are identical with the epithelioid cells of the arteriovenous anastomoses studied by Hoyer, Vastarini-Cresi, Grosser, Schumacher, Masson,[7] Clara,[8] Grant[9] and Popoff.[10] These complex arteriolar formations control the local circulation of blood (Masson[7]). By analogy, the identical structure of the juxtaglomerular apparatus suggests a similar function; i. e., the afibrillar leiomyoblasts, situated between a contractile arteriolar segment and capillary network must intervene in the eventual exclusion of the glomerular loops from the renal circulation. When this occurs, the blood can be diverted along aglomerular arteriolar shunts, the importance of which is still under discussion (Dehoff[11]; Oliver and Luey[12]).

According to our observations on the dog, in a vascular unit composed of an interlobular prearteriole and its branches, at least one aglomerular arteriole communicates directly with the intertubular capillaries. This shunt acts as a safety valve when all the glomeruli of the unit are excluded from the circulation. We do not think that these shunts are as numerous as was admitted by Dehoff, and our statement is supported by the observations of Oliver[13] in the normal human kidney.

How do the epithelioid cells of the arteriovenous glomi and of the juxtaglomerular neuromyoarterial apparatus in particular control the course of the blood? Their turgescence should close, respectively, the arteriovenous shunts or the access of the blood to the glomerulus, according to Clara.[8]

If Clara's contention is correct, what is the meaning of the isolated afibrillar cells found along the wall of the efferent glomerular arteriole, where their turgescence cannot close the lumen? Neither does the swelling of the subintimal epithelioid cells of the interlobular prearteriole serve any physiologic purpose. It must be borne in mind that the arteriovenous shunts and glomic structures contain practically always contractile elements in their walls (Masson[7]).

It seems reasonable to admit that the smooth muscle cells of the vas afferens and not the afibrillar cells are responsible for the narrow-

7. Masson, P.: Les glomus neuro-vasculaires, in Policard, H.: Histophysiologie, Paris, J. Hermann, 1937.
8. Clara, M.: Ergebn. d. Anat. u. Entwcklngsgesch. **27**:246, 1927.
9. Grant, R. T.: Heart **15**:281, 1930.
10. Popoff, N. W.: Arch. Path. **18**:295, 1934.
11. Dehoff, E.: Virchows Arch. f. path. Anat. **228**:134, 1920.
12. Oliver, J., and Luey, A. S.: Arch. Path. **18**:777, 1934.
13. Oliver, J.: Arch. Path. **20**:962, 1935.

ing of the lumen and the eventual exclusion of the glomerular loops from a circulation.

Taking into account the richness of the nerve supply of the glomi of the skin and observing a similar, though scantier, network around the juxtaglomerular apparatus on sections stained by nonspecific trichromic staining method, Goormaghtigh [1] ventured the hypothesis that from the clear afibrillar cells could originate a reflex controlling the contraction or the relaxation of the smooth muscle cells of the arteriole. Masson [7] suggested a similar mechanism in his last paper. However, the mode of innervation of the arterioles is still under discussion. In accordance with Stoehr [14] and Boeke,[15] who examined organs other than the kidney, we have seen an intricate sympathetic plexus spread over the outer surface of the renal arterioles. Along this network the nervous impulse can be conducted in various directions from one arteriolar segment to another; i. e., a stimulation registered by juxtaglomerular or isolated paucifibrillar cells could be transmitted to more distant contractile elements as a local reflex. Hirt [16] stated that myelinated fibers reach the kidney by way of the minor splanchnic nerve. Using the Kulschitzky hematoxylin method, however, we did not find them along the arterioles of a normal kidney that had remained for more than a year in a 3 per cent solution of potassium bichromate. It is doubtful if myelinated nerve fibers are connected with the arterioles of the renal cortex. Von Smirnow [17] found sensory nerve endings in the wall of the origin of the interlobular artery and of the interlobular vein. A spinal reflex arc originating in the arterioles of the renal cortex is thus questionable.

The investigations of Clark and his associates [18] with the transparent chamber technic give important information about the function of the afibrillar myoblasts: The arteriovenous anastomoses of the rabbit's ear contract spontaneously; the rhythm of the contractions is independent of the pulse rate (from 4 to 6 a minute). In the main the rhythmic contraction of the arterioles is slower. The different arteries and different parts of the same artery under observation each contract at a different tempo. Rhythmic contractions of the glomerular arteriole were observed long ago by Nussbaum and Policard [19] in lower vertebrates. Since the wall of arteriovenous anastomoses contains chiefly

14. Stoehr, P., in von Möllendorf, W.: Handbuch der mikroscopischen Anatomie des Menschen, Berlin, Julius Springer, 1927.

15. Boeke, J.: Ztschr. f. mikr.-anat. Forsch. **35**:551, 1934.

16. Hirt, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **78**:260, 1926.

17. von Smirnow, A. E.: Anat. Anz. **19**:347, 1901.

18. (*a*) Clark, E. R., and Clark, E. L.: Am. J. Anat. **54**:229, 1934. (*b*) Clark, E. L., and Williams, R. G.: ibid. **55**:47, 1934.

19. Nussbaum and Policard, quoted by Bouin, P.: Eléments d'histologie, Paris, Félix Alcan, 1932.

afibrillar or paucifibrillar cells, it is almost certain that the latter are in some way responsible for the spontaneous active rhythmic nature of the contractions. On the other hand, the arterioles of the rabbit's ear contain a much smaller number of these particular cells; this would account for the slower rate of the rhythmic contractions of the arterioles noticed by Clark. According to Sandison,[20] the contraction appears first in the main artery of the ear and extends as a wave along the arteries and their branches and the arterioles. This implies the possible, if not the real, existence of a diffentiated tissue responsible for the conduction of this contraction along the wall of the vessel.

The physiologic properties demonstrated by Clark and his collaborators and the recent observations of Wybauw[21] on arteries tend to show that the heart, arteriovenous anastomoses, arteries and arterioles have in common, to a variable extent, excitability, rhythmic contractility and a mode of conduction of the contraction wave. The nodal tissue and His bundle assume the function of conduction in the heart. We believe that the anatomic substratum of the latter finds its counterpart in the afibrillar or paucifibrillar leiomyoblasts of the arterioles, which also tend to aggregate in nodes (neuromyoarterial) and bundles. As in the heart, the acquirement of the prevalent functions of conductibility and excitability corresponds to a complete or partial loss of myofibrils. However, as in the heart, this scarcity or absence of myofibrils is not an indispensable morphologic feature for the function of conduction; transitional types with fairly abundant myofibrils occur in the heart, arteries and arterioles; no specific tissue joins the node of Keith and Flack to the node of Tawara. Yet the wave of contraction goes on, uninterrupted, from the region of the sinuses to the point of the heart. In renal arterioles interruptions are more numerous. There are other important differences: The rhythm of arteriolar contractions is more erratic and liable to minor local interferences. This implies a greater excitablity of the arteriolar afibrillar cells and probably a more important interference of the innervation. In fact, Clark, Clark and Williams[18b] observed rhythmic contraction only when the arterioles were innervated. The continuous changes in tempo of the rhythmic contraction are best accounted for by occurrence of local reflexes. In this case, the afibrillar cells would be the starting point of these reflexes and would have to some extent a sensory function. They would also conduct the contraction wave. They may form the link between the periarteriolar nerves and the contractile smooth muscle cells.

We do not know yet what influences the afibrillar leiomyoblasts in the kidney. The subintimal position of many of them (fig. 1 *A, B* and *D;* fig. 2 *A*) implies possible intervention of variations of the endo-

20. Sandison, J. C.: Anat. Rec. **54**:105, 1932.

21. Wybauw, R.: Bull. Acad. roy. de méd. de Belgique **15**:604, 1935.

vascular pressure (compare observations on isolated arteries by Bayliss,[22] Wybauw [22] and Hess [22]) or of the physicochemical constitution of the blood. In this respect, the absence of a subintimal elastic membrane in the neuromyoarterial apparatus is significant. The afibrillar leiomyoblasts which lie on the surface of the arterioles (fig. 1 *C*; fig. 2 *B*) could be influenced by changes in caliber of the surrounding capillaries or tubuli. This assumption is supported by the experiments of Okkels and Peterfi,[23] who observed that the vas efferens contracts when touched by the needle of the micromanipulator. Our morphologic observations, interpreted in the light of Clark's experimental work, show that the arteriolar segments are built on a pattern analogous to that of the heart and evoke Senac's old aphorism: *"les artères sont de vrais coeurs sous une autre forme"* (the arteries are true hearts in another form). Our contribution is in favor of the still much discussed theory of the relative functional individuality of the peripheral arterioles, inaugurated by Haesebroeck.[24] A full discussion of arterial and arteriolar tone will be found in a recent report of Heymans and Brouha.[22]

Observations on the Smooth Muscle Cell.—As Boeke [25] remarked, the problem of the structure of smooth muscle cells contains many unsolved riddles. We deal here only with facts which stand in direct relation to our subject.

In the afibrillar area of the protoplasm there frequently are vacuoles. These are more conspicuous in the media of the interlobular prearteriole than in the vas afferens. They are numerous at the origin of the glomerular arteriole. They contain a colorless material which shows none of the reactions of fats or lipoids. The vacuoles are formed around juxtanuclear fuchsinophil bodies (fig. 2 *C* and *E*); first a clear halo surrounds them; later the fuchsinophil body fades away. The formation of vacuoles corresponds to one of the stages of the cell metabolism; the vacuoles are eliminated in the loose connective tissue surrounding the vessel. The arteriolar wall is no exception to the rule; its interstitial fluid is dependent on the specific activity of its cellular constituents.

ARTERIOLAR HYPERTROPHY

The observations discussed in this section correspond to those on the dogs of table 1 and have been checked over and over again on a considerable number of preparations. After each experiment several camera lucida drawings of arterioles of one kidney were compared with an equivalent number of drawings of normal renal arterioles. Comparison

22. Cited by Heymans, C., and Brouha, L., in Le spasme vasculaire, Journées internationales de cardiologie, Clermont-Ferrand, Imprimeries de l'Avenir, 1937, p. 1.

23. Okkels, H., and Peterfi, T.: Ztschr. f. Zellforsch. u. mikr. Anat. **9**:327, 1930.

24. Haesebroeck, P.: Die Blutdrucksteigerung, Wiesbaden, J. F. Bergmann, 1910.

25. Boeke, J.: J. Comp. Neurol. **56**:27, 1932.

was always made between corresponding segments of glomerular arterioles or interlobular prearterioles. Notice was taken of the smaller size of the arterioles of the periphery of the cortex as compared with those situated near the medulla. Of this important graphic documentation, a drastic selection had to be made for publication (fig. 2 *C, D, E, F* and *G*.).

Hypertrophy of the Media.—In all the animals of table 1 both types of cells of the media were hypertrophic. This is proved by a comparison of *C* and *D* with *A* and *B*, corresponding to normal dogs, in figure 2. The size of the smooth muscle cells of dogs 19 (fig. 2 *D*) and 13 (fig. 2 *C*) is striking. *C* and *D* show also the hypertrophy of the afibrillar cells of the neuromyoarterial apparatus and of those found along the wall of the arteriole. A remarkable hypertrophy of the neuromyoarterial apparatus of dog 19 is seen in figure 3. The latter bulges into the glomerular chamber. However, all the cells of the media are not hypertrophic; this gives a tortuous outline to the longitudinal sections of arterioles (fig. 2 *C* and *D*), which do not lend themselves to accurate measurements. Only reconstruction methods could express this hypertrophy numerically. The marginal myofibrils, although staining vividly, are separated from one another by a wider space as the result of the cell's turgor. The nuclei are hypertrophic (in fig. 2, compare *A* and *B* with *C* and *D*). The stimulating effect of calciferol is also proved by the abundance of binuclear cells (fig. 2 *F* and *G*); a process of nuclear strangulation takes place; we never saw a mitotic division. The binuclear cells belong as much to the fibrillar as to the afibrillar type. Normally, the presence of two nuclei is exceptional in smooth muscle cells but is fairly frequent in the afibrillar cells of the media. In figure 2, *G* presents a binuclear afibrillar cell: the chromaticity and comparatively small size of the two nuclei in close contact suggest that nuclear division has just taken place. The figures referred to correspond to arterioles, but we could demonstrate the same features in the interlobular prearteriole. Hypertrophic cells were encountered also in most of the dogs of the other series (tables 2 and 3), where arteriolar degenerative changes were already manifest (for instance dog 24). Figure 4 represents an afibrillar leiomyoblast with three nuclei in the wall of a capsular arteriole of the adrenal of a dog whose renal arterioles were severely damaged. In figure 5, *C* shows hypertrophic and degenerated contractile smooth muscle cells intermingled. Binuclear smooth muscle cells appear also in recuperating arterioles (dog Thér.).

Vacuolation.—Vacuoles were observed in abundance in the arterioles of the dogs of table 1 (fig. 2 *C*). Such vacuoles are not a sign of regression, since they are found in normal specimens. Their contents

are eliminated in the periarteriolar space and also, in contrast with our controls, under the internal elastic membrane. As a result of extensive vacuolation the space between the muscular cells enlarges; vacuolation is always more marked in the interlobular prearteriole than in the afferent and efferent glomerular arterioles. It is most marked at places of bifurcation.

Increase of Fuchsinophil Bodies.—In close relation to exaggerated vacuolation stands the increase of fuchsinophil bodies (figs. 2 *C* and *E*; 5 *A*, *C* and *H*); the latter are frequently found in binuclear cells (fig. 5 *A*) or close to a nucleus in the

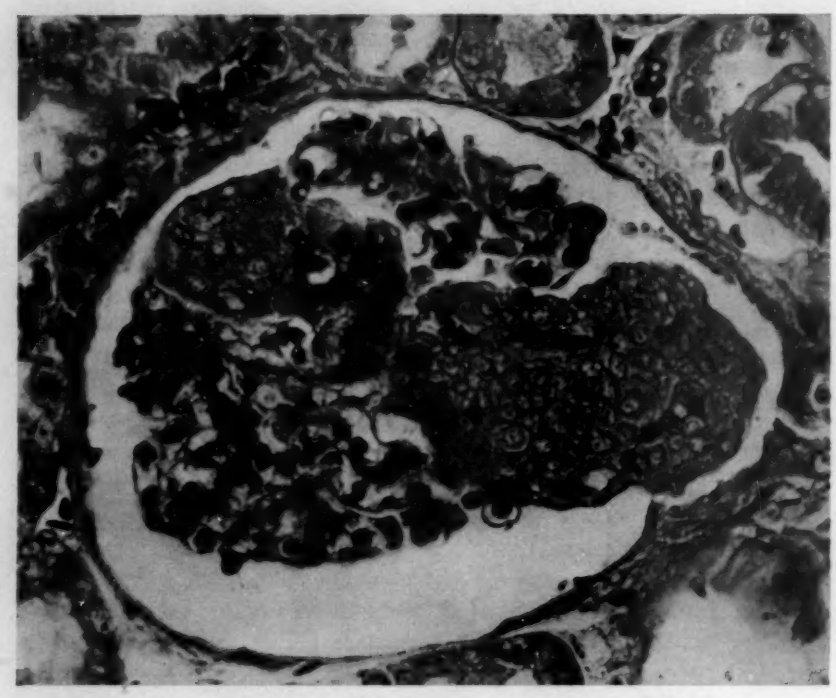

Fig. 3 (dog 19).—Considerable hyperplasia of the juxtaglomerular neuromyoarterial apparatus, leading to an invasion of the glomerular tuft; × 550.

process of amitosis. In dogs 3, 4, 21 and 22 they were rarely surrounded by a halo, but in dogs 11, 12 and 19 their intervention in the formation of vacuoles cannot be doubted. This cytologic difference suggests that the metabolism of the cells of the media in dogs 3, 4, 21 and 22 was at a different stage than that in dogs 11, 12 and 19 and was influenced by the dosage.

Periarteriolar Edema.—The increased vacuolation and the elimination of more waste products are in all probability responsible for the perivascular edema and the intensified histiocytic or plasmocytic reaction (fig. 5 *A*).

Comment.—Since these changes are only an exaggeration of what is seen in the normal, we consider them as morphologic evidence of

increased metabolism in the cells of the media, owing to stimulation by calciferol. No toxic effect on the arterioles could be found in this series (table 1). A comparative review of the dogs of table 1 tends to show that the stimulative effect of calciferol on the arterioles was slight or incipient in dogs 1, 2, 3, 4, 21 and 22, given comparatively small doses; that this effect was marked but irregularly distributed in dogs 10, 11, 12 and 13, given a larger dose in a shorter time, and was generalized and striking in dog 19, given daily doses of 110 micrograms per kilogram during one hundred and fourteen days.

REGRESSIVE ARTERIOLAR CHANGES

When vitamin D is administered in larger doses and for a longer period than indicated in the previous section, the smooth muscle cell undergoes regression and even necrosis (tables 2 and 3). Our aim

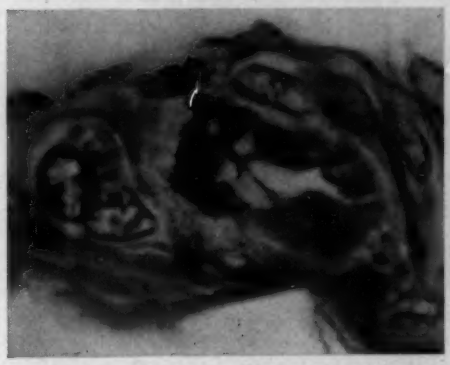

Fig. 4 (dog 24).—Trinuclear afibrillar leiomyoblast in an arteriole of the capsule of an adrenal; × 1,600.

is to show that the injury to the cell is the outcome of overstimulation by demonstrating the links between the hypertrophic and the regressive stages. The regular progression of cell alteration excludes any misinterpretation concerning its experimental character.

Spontaneous Lesions.—Arteriolar or prearteriolar lesions were never found in our young adult controls. It is known that in old dogs these vessels are occasionally involved. Gross lesions of the tubules were absent in our controls, but the lipoid content of the proximal Henle loop varied to some extent. The glomeruli were normal. A conspicuous feature in many control dogs was the presence of lymphoid infiltration showing an inconstant localization in the cortex or in the medulla or in both at once. These lymph cells tended to form round cell masses, resembling in some way lymph follicles; their centers were composed of large clear cells surrounding a larva of a worm. These infiltrations, which were generally discrete and caused no marked changes in the adjoining tubules, were found in 53 per cent of our controls and in 50 per cent of the treated dogs whose arterioles showed only arteriolar hypertrophy.

Resistance of Afibrillar Cells to Injury.—Contrary to smooth muscle cells, the afibrillar, probably sensory leiomyoblasts, withstand large doses of vitamin D.

In dogs 23, 24, 25 and Bas. (table 2), the glomerular arterioles were severely damaged (fig. 5 *B*, *D* and *E*), but the afibrillar cells and the neuromyoarterial segment were intact or sometimes hypertrophic (fig. 5 *B*). Lethal doses are needed to bring the afibrillar myoblasts to regression (condensation of chromatin and vacuolation), and even then the degenerative process is not generalized. In short, the administration of vitamin D in appropriate doses discriminates the afibrillar cells from the ordinary muscle cells (fig. 5 *E*).

Regressive Changes of Smooth Muscle Cells.—Degeneration does not occur simultaneously or with the same intensity in all arterioles. In the liver, the smooth muscle cells begin to regress only after the administration of an amount of calciferol that kills the animal in from seventy-four to one hundred and thirty hours and produces, in this short space of time, extensive necrosis of the media in the kidney, spleen, thyroid and pancreas. On the other hand, the cell alterations are not uniformly distributed in one particular arteriole. Only the distal segments of the interlobular prearterioles of dogs 23, 24 and 25 were severely damaged. This lack of synchronism when moderate doses are used provides a histologic picture lending itself to the study of the successive degenerative changes.

Figure 5 *C* corresponds to a tangential section of the proximal end of an interlobular prearteriole of dog 23. Three types of cells are to be noted: (*a*) The cell to the left is hypertrophic and contains distinct myofibrils. The nucleus of the cell to the right is large, but the myofibrils tend to vanish; a juxtanuclear fuchsinophil body characterizing the hypertrophic stage is present. (*b*) Another group of cells shows different stages of vacuolation; the myofibrils are still visible in one of the cells but not in the others. A large vacuole fills one of them without impairing the condition of the nucleus, but in the adjoining vacuolated cell the nucleus has a compact structure. (*c*) Two other cells are distinctly atrophic, but the nuclear chromatin network is preserved; the myofibrils are normal in one of them; in the other they are coarser and irregularly stained with iron hematoxylin. The function of these cellular elements is not irrevocably impaired.

In other segments of the same prearteriole, however, degeneration is much more advanced, as shown in figure 5 *D*; the changes represented in figure 5 *C* precede those illustrated in figure 5 *D*. By comparing the two figures, it is possible to realize what becomes of each of the three types of cells (*a*, *b* and *c*) analyzed in figure 5 *C*.

In severely damaged arteriolar segments the hypertrophic nonvacuolar cell (*a*) loses its myofibrils; the size of its nucleus is reduced; as the process of regression goes on, the chromatin vanishes and the nuclear membrane stains unevenly. We call this regressive stage clear tumefaction (see clear large cell of fig. 5 *F*; also fig. 5 *D*). What becomes of the vacuolar cells (*b*) of figure 5 *C*?

The large vacuoles are not expelled, and their contents solidify. The nucleus shrinks, stains intensely but does not attain the pyknotic stage. The atrophic stage (*c*) (fig. 5 *D*), on the other hand, leads to cell shrinkage. The protoplasm stains vividly with acid dyes; what remains of the myofibrils appears first as coarse black rods (iron hematoxylin), which break up later into irregular lumps (fig. 5 *D*). This type is labeled "shrunken cell" (see small cells of fig. 5 *B* and *F*). A comparison of dogs 19, 23 and 24 shows that the nucleus becomes pyknotic (see the necrotic cells in fig. 5 *B, D, E* and *G*) only at the end of the regressive process. In chronic experiments, the basophil fluid of the pyknotic nucleus runs out into the cellular area and even into the intercellular spaces under the internal elastic membrane or along the outer surface of the media (fig. 5 *G*).

In short, at the end of the period of stimulation by vitamin D the smooth muscle cells are either hypertrophic, abnormally vacuolated or normal. If then the calciferol treatment is continued, the cellular hypertrophy leads to clear tumefaction; the hypervacuolation, exaggeration of a normal process, impairs, in the long run, the nuclear structure, while the cell with normal features undergoes atrophy which, in turn, may lead to shrinkage and necrosis. In experimental material, pyknosis and necrosis may be rare or absent, while the other signs of regression are marked (table 3).

When the dosage and the duration of the experiment correspond to those indicated in table 2, the media has a polymorphic appearance (fig. 5 *D*). In more severe or protracted dosage, the hypertrophic type subsides, while all the other gradations of regression are still present (table 3). The necrotic cells disappear slowly. In dog Thér., a year after the calciferol treatment was discontinued, these cells were still present in fair numbers (fig. 5 *H*). Whether these cells were already necrotic at the end of the calciferol treatment or whether some shrunken cells subsequently turned necrotic is a question difficult to answer. We had hoped to obtain a process of hyalinization of the media by allowing some of our animals to survive for some time after the calciferol feeding. However, the animal that survived more than a year (Thér.) showed only a discrete deposit of collagen between the muscle cells of the interlobular prearteriole. This observation answers to a certain extent the question raised by Cowdry and Scott [26] about the progressiveness of the arteriolar lesions in monkeys fed viosterol. Perhaps a longer period of calciferol administration or even a longer period of survival might produce scarring of the media of small arteries.

In the animals referred to in this section, patient investigation revealed only now and then a split internal elastic membrane. Contrary to what has been observed in the rabbit, calcification of the media, sought for by the Kossa method, never occurred. With comparatively small doses, Cowdry and Scott [26] did not notice calcification in Macacus rhesus either. Calcification is not the principal feature in the arteriolar pathologic picture of hypervitaminosis D but only an epiphenomenon.

26. Cowdry, E. V., and Scott, G. H.: Arch. Path. **22**:1, 1936.

Distribution of Regressive Arteriolar Lesions.—The interlobular prearteriole is the most sensitive of all renal vessels. This is also true of prearterioles of other organs. Its distal end degenerates first, and its subintimal muscle cells show the severest alteration (fig. 5 *D*). The places of bifurcation are weak points; intense vacuolation precedes actual regression (fig. 5 *D*). The sensitiveness to injury of the interlobular prearteriole is known in human histology (Russell [27]).

The glomerular arterioles resist the noxious agent longer than the interlobular prearteriole. The latter may already show regressive signs while the vas afferens is still normal or hypertrophic (dog Pros.). The distribution of the lesions is segmentary or focal, and often one particular segment is at the same stage of regression. Small segments of the vas afferens escape injury. These observations corroborate the notion of functional individuality of arteriolar segments emphasized by Clark and his associates [18] and earlier still by Ricker.[28]

As the result of medial degeneration, the lumen of the arteriole is wide: The arteriole looks like a venule. Arterioles of the same caliber in the spleen undergo similar changes. Later, degeneration sets in also in arterioles of the pancreas, neurohypophysis, thyroid and adrenal.

Endothelial and Perivascular Changes.—The vacuolation of the endothelial cells of the arterioles, noticeable in normal specimens, is exaggerated after prolonged administration of calciferol; the endothelial nuclei of arterioles and capillaries enlarge, and mitotic figures are found occasionally (arterioles of dog Bas., table 2; capillaries of dog Phil., table 3). The severe degeneration of the media of the arterioles does not interfere with the morphologic aspect of the intima. When the animal dies during or after a protracted period of heavy dosage with calciferol, and when severe Kupffer cell reaction in the liver, monocytosis, pronounced excretion of calcium (see page 1164 of this paper) and interstitial sclerosis of the kidney are evidence of the toxic effect of the drug, discrete intimal infiltrations are observed exceptionally at the origin of the interlobular prearterioles (dog Jean, table 3). In the larger visceral arteries of this dog we observed also incipient changes of the intima which were identical with the early stages of human arteriosclerosis. But these arteries are not considered in this paper.

As during the hypertrophic stage, we notice periarteriolar edema; histiocytic and plasmocytic infiltration increases slightly, especially near the places of bifurcation. The perivascular connective cells enlarge considerably when large doses are used (dog Phil., table 3).

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27. Russell, D. S.: A Classification of Bright's Disease, Medical Research Council, Special Report Series, no. 142, London, His Majesty's Stationery Office, 1929.

28. Ricker, G.: Sklerose und Hypertonie der innervierten Arterien, Berlin, Julius Springer, 1927.

Evidence of Recovery of Smooth Muscle Cells.—The process leading to necrosis of the smooth muscle cell is marked by a succession of regressive stages. When the animal is killed a few weeks after the treatment has been discontinued, the tumefied, atrophic and shrunken muscle cells gradually decrease in number (dog Bas., table 2; Yol., Jean, Char., Thér., table 3). The arterioles tend to recover their former aspect; only the necrotic, laked cells remain. Figure 5 *H* represents a glomerular arteriole of a dog treated during a month and surviving four hundred and twelve days; the animal had been pregnant in the meantime. A comparison with figure 5 *D* and *E* from dog 24, killed soon after it had received the last dose of calciferol, gives conclusive evidence of the existence of a recovering process in the arteriolar media.

Comment.—Studies on hypervitaminosis D in the dog offer a good approach to arteriolar pathologic conditions which, as Plaut [29] recently remarked, deserve a much closer attention than they have received so far.

In the past, lesions caused by vitamin D have been investigated chiefly in the rabbit and, though the distribution and localization of the gross arterial lesions have been recognized, the interest of our predecessors has been focused on the aorta and great arteries (Schmidtmann; [30] Hückel and Wenzel; [31] Laas; [32] Schiff; [33] Huebschmann [34]).

The rabbit is not the most suitable animal for the study of vascular diseases because the structural changes succeed each other at too quick a pace, and extensive calcinosis masks the primary lesions. In the dog the vascular changes reach their full development at a much slower tempo. In the rabbit the whole vascular system is involved at the same time, while in the normal dog no modality of calciferol administration injures the aorta macroscopically; even the microscopic changes are so slight that their detection requires patient investigation.

Our experimental observations show the distinct behavior of the aorta, on one hand, as compared with that of the arteriolar and prearteriolar system, on the other. This different reaction to calciferol (vitamin D_2) reminds us of the opposition often observed in man between arteriosclerosis of the aorta and the large arteries (central arteriosclerosis) and arteriolar sclerosis (peripheral arteriosclerosis of Jores).

In the aorta of the rabbit calciferol is supposed to damage, in the first place, the elastic tissue (Tatsuji [35]). How far this is true for the aorta

29. Plaut, A., in discussion on Otani, S.: Arch. Path. **16**:435, 1933.

30. Schmidtmann, M.: Virchows Arch. f. path. Anat. **278**:408, 1930.

31. Hückel, R., and Wenzel, H.: Arch. f. exper. Path. u. Pharmakol. **141**: 292, 1929.

32. Laas, E.: Virchows Arch. f. path. Anat. **278**:346, 1930.

33. Schiff, A.: Virchows Arch. f. path. Anat. **278**:62, 1930.

34. Huebschmann, P.: Beitr. z. path. Anat. u. z. allg. Path. **84**:251, 1930.

35. Tatsuji, I.: Mitt. a. d. path. Inst. med. Fac. Niigata, Japan **46**:1, 1936.

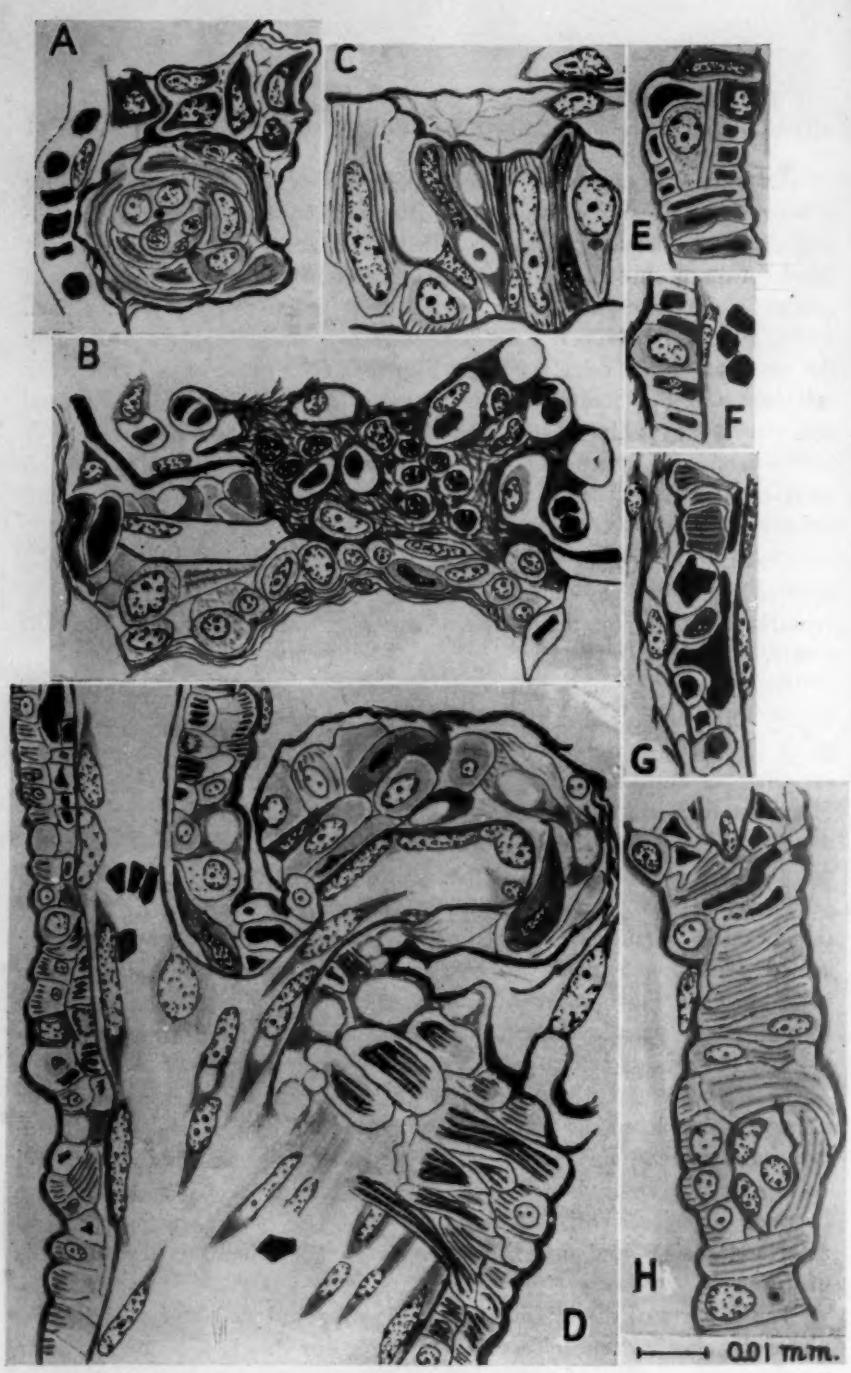

Fig. 5.—*A* (dog 3), fuchsinophil body in a binuclear cell; periarteriolar histiocytic reaction. *B*, (dog Bas.), vascular pole of a glomerulus, showing two necrotic smooth muscle cells contrasting with hypertrophic afibrillar myoblasts. *C* (dog 23), segment of interlobular prearteriole (tangential section), showing hypertrophy, vacuolation and periarteriolar edema. *D* (dog 23), various regressive cellular changes at the point of bifurcation of an interlobular prearteriole. *E* (dog 23), vas afferens, showing intact subendothelial afibrillar cell surrounded by shrunken and necrotic smooth muscle cells. *F* (dog Chris.), clear tumefaction of a smooth muscle cell. *G* (dog Bas.), basophil substance running out under the internal elastic membrane. *H* (dog Thér.), recuperating cells adjoining necrotic cells.

of the dog will be examined in another paper. In the arterioles of the dog calciferol leaves the scanty elastic membrane intact but acts, in the first place, on the two cellular components of the media: the contractile smooth muscle cell and the afibrillar leiomyoblast endowed with physiologic properties probably analogous to those of the nodal tissue of the heart. Judged by morphologic criteria, calciferol in small daily doses stimulates both types of cells just as it increases the metabolism of other tissues.

Handovsky [36] found that oxidation in hepatic tissue was increased in animals which were given vitamin D_3. Szittyay,[37] working in our pathologic department, observed a gradual and intense increase of the binuclear Purkinje liver cells of the rabbit during the administration of irradiated ergosterol (in the form of vigantol). With a heavier dosage in the present study, we observed mitoses in the reticuloendothelial cells of the liver and in the endothelial lining of the capillaries of the kidney. On the other hand, vitamin D favors the repair of wounds.

In arterioles and prearterioles this stimulation is recognized by cell hypertrophy. The noxious effect of overdosage or of too protracted treatment can be the result of too strong or too prolonged stimulation. In other words, hypertrophy of the contractile smooth muscle cell precedes, in most instances, its degeneration.

It must be emphasized that in hypervitaminosis D the afibrillar cells become hypertrophic but practically never degenerate. Would this form the anatomic basis for an explanation of the increased excitability of the arterial wall observed by Wybauw [38] in human hypertension? At any rate, the administration of calciferol dissects, as it were, the two components of the media and proves that they react differently, an indication that they may have a different function. The resistance of the afibrillar cells to the noxious effect of vitamin D reminds us of Popoff's [10] observations. This author found that the wall of the Sucquet-Hoyer canal of the cutaneous glomi, composed of afibrillar epithelioid cells, was not primarily involved in senile arteriosclerosis or in thromboangiitis obliterans.

Arterial and arteriolar hypertrophy occupies a prominent place in the pathology of hypertensive diseases (Volhard and Fahr; [39] Schuermann and McMahon [40]). Its significance is the subject of endless controversy. We have in vitamin D a biologic substance which, given in adequate doses, causes almost simultaneously hypertension [41] and hypertrophy of the arteriolar media (tables 1 and 2).

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36. Handovsky, H.: Arch. f. exper. Path. u. Pharmakol. **159**:383, 1931.
37. Szittyay, Z.: Compt. rend. Soc. de biol. **124**:296, 1936.
38. Wybauw, R.: Mém. couron. Acad. roy. de méd. de Belgique **23**:1, 1928.
39. Volhard, F., and Fahr, T.: Die Brightsche Nierenkrankheit: Klinik, Pathologie und Atlas, Berlin, Julius Springer, 1914.
40. Schuermann, P., and MacMahon, H.: Virchows Arch. f. path. Anat. **291**: 47, 1933.
41. Handovsky, H.: J. Physiol. **90**:1262, 1937.

By increasing the dosage we could produce at the same time, in different arteriolar sections of the kidney, hypertrophic and regressive changes which had the same distribution as in malignant or even benign nephrosclerosis in man. The noxious agent of nephrosclerosis has in common with vitamin D the fact that in the initial stage of its action it causes hypertension and arteriolar hypertrophy which lead to regressive changes as time goes on. In man, as in the dog, the degeneration or necrosis appears, in the first place, at the prearteriolar bifurcations (Russell [27]), and afterward in the arterioles themselves. Unless large lethal doses are used, the arterioles never degenerate in their whole length; well outlined segments escape injury.

In other respects, of course, even when we limit our discussion to the vascular system, a wide gap persists between our results and the observations made in human vascular nephrosclerosis. We can do no more at present than to look for analogy and not for identity.

In the meantime, it is obvious that identical changes can be found only in acute diseases. No systematic work has been done in this direction as far as we know. It will be a slow task, because the detection of the lesions we have described needs perfect fixation of fresh material; these conditions are rarely fulfilled.

With these considerations in mind, we have examined freshly fixed kidneys from persons who had acute hypertensive diseases, such as eclampsia gravidarum and acute scarlet fever; we have also examined kidneys from persons who had toxic diphtheria with marked uremia. We can state that in eclampsia and scarlet fever the arteriolar lesions resemble very closely those of hypervitaminosis D in the dog.[1] In toxic diphtheria, when no rise of the blood pressure was observed, there was one slight difference: While shrinkage or necrosis of the contractile smooth muscle cells of the prearterioles was marked, the hypertrophy of the afibrillar myoblasts was less evident. It seems as if the hypertrophic reaction of the afibrillar cells is more closely related to the occurrence of hypertension than the hypertrophy of the smooth muscle cells.

These observations give a new stimulus to the study of pathologic changes in the arterioles. Vitamin D_2, lately rather neglected as an experimental agent in the study of vascular changes, can be of great help in this new direction.

TUBULAR AND INTERSTITIAL CHANGES

The kidneys of the dogs reviewed in this paper must be divided into two groups; in one, the tubules remain almost normal (tables 1 and 2); in the other, the Henle loops, the distal convoluted segments, the intercalated segments and the collecting tubes are altered and, more often than not, distended to a variable extent (table 3); they are filled with hyaline and calcium casts. If abundant and large, these casts are

responsible for the tubular distention clearly demonstrated in figure 6 *A* and noticeable even after a short interval when a unique very large dose (9 mg. per kilogram) of calciferol has been administered (dog Louis). The calcium precipitation is the result of an increased discharge of cal-

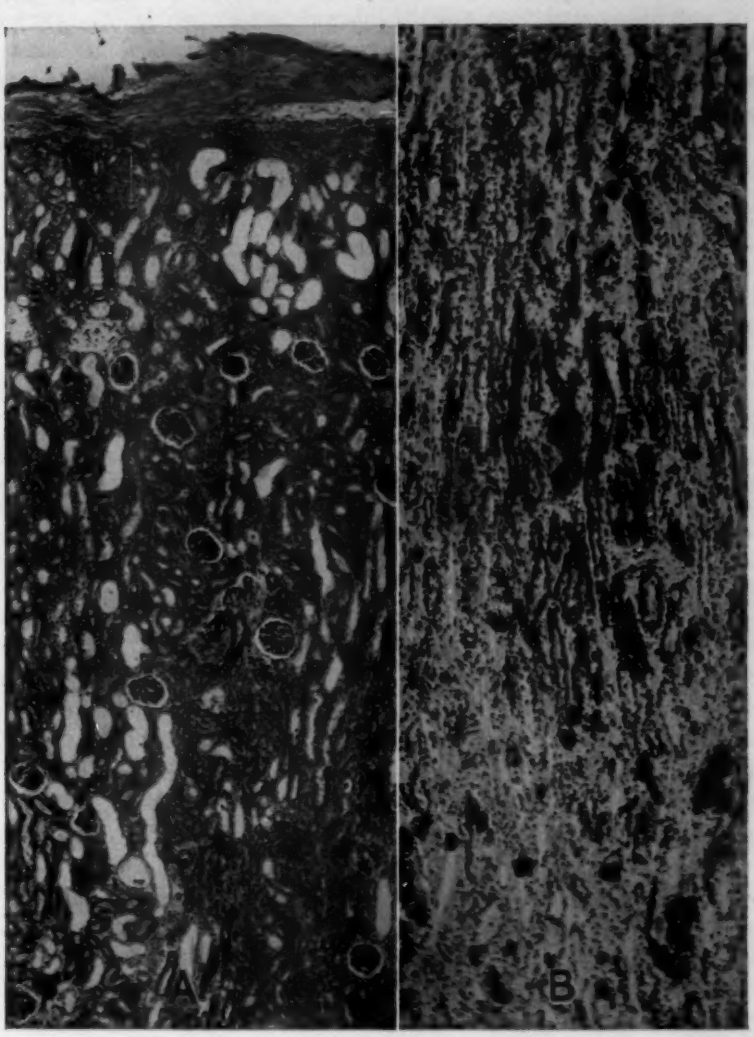

Fig. 6.—*A* (dog Guil.), tubular distention. The glomeruli connected with the distended tubuli are collapsed. *B* (dog Jean), Kossa preparation; × 40.

cium phosphates through the glomerular tufts and also of faulty resorption of calcium phosphates by the distal segments of the nephron. The perfect gradation of the epithelial lesions proves that there is this defect in the tubular resorption process. After the administration of small

daily doses, minute calcium casts are occasionally found to be present in the renal papilla without causing distention (table 2). There is no apparent cell destruction. With larger doses, given at regular intervals, the casts become more conspicuous, but the epithelial lesions remain discrete, and the general aspect of the tubes remains normal. The presence of regenerative mitosis points, however, to a previous tubular damage. The glomeruli are normal; no albuminuria and few calcium casts are observed.

Taylor, Weld and Sykes [42] suggested that after administration of parathyroid extract or of vitamin D, the blood calcium diffuses easily and noticed that the increase in calciuria appears later than the rise in the blood calcium. These authors concluded that the calcium phosphate which has filtered through the glomerulus is reabsorbed during the initial period of the administration of vitamin D. In our view, the calcium casts indicate a failure of the tubules to reabsorb the filtered calcium salts, and their presence coincides with epithelial lesions, however discrete.

We must refer here to the clinical observations of Albright and Bloomberg [43]: In hyperparathyroidism, comparable in many respects to hypervitaminosis D in the dog, the presence of calcium casts in the urine is a constant feature; the urine contains no albumin or only the slightest trace; the absence of albuminuria implies in all probability the integrity of the glomeruli. As the urine becomes less acid, the granular calcium casts gradually disappear; this points to a mild alteration of the renal parenchyma. There are no irreparable organic lesions in these mild conditions which can be compared with those of dogs treated with small doses (from 50 to 230 micrograms per kilogram) or with larger doses of calciferol (from 500 to 600 micrograms per kilogram) for a short period.

While still larger doses (table 3) are given, the tubular resorption of calcium fails, and large calcium casts (fig. 6 *B*) collect, chiefly in the ascending Henle loops (Gough,[44] in observations on the rat) but also in more distal parts of the nephron, close to the renal capsule or in the collecting tubes of the papilla (Cowdry and Scott [26]). The amount of calcium in these casts estimated by histochemical methods (Kossa; hemalum) varies from one place to another. In the distal convoluted segments and the cortical segments of the collecting tubes the casts attain a considerable size but contain less calcium. If treatment is discontinued for some time, the Kossa method often fails to reveal calcium in the casts; a gradual resorption of calcium must take place.

These casts (protein + calcium or protein — calcium) cause intense tubular distention and accentuate the initial epithelial destruction. Giant cells appear in the lumen of the tubule. The basement membrane thickens and becomes impregnated with calcium (fig. 6 *B*). The collecting tube and the distal convoluted segment shrink and undergo atrophy.

42. Taylor, N. B.; Weld, C. D., and Sykes, J. F.: Proc. Roy. Soc., London, s.B **116**:10, 1934.

43. Albright, F., and Bloomberg, E.: J. Urol. **34**:1, 1935.

44. Gough, J.; Duguid, J. B., and Davies, D. R.: Brit. J. Exper. Path. **14**: 137, 1933.

The ascending Henle loop is destroyed. It is remarkable that the proximal convoluted tubule escapes injury. The regressive changes of the distal segments of the nephron and the imbibition of the intertubular spaces by calcium salts call forth an intense plasmocytic reaction and the formation of granulation tissue (fig. 10). Along the collecting tubes, distal convoluted segments and ascending Henle loops, sclerosis sets in. Since the distal convoluted segment always comes in close contact with the vascular pole of the glomerulus (Peter; Zimmermann;[5] Oliver and Luey;[12] Goormaghtigh;[6] fig. 7), it is obvious that the vas afferens will be surrounded in many places by inflammatory or connective tissue (fig. 10). When the animal survives the period of calciferol administra-

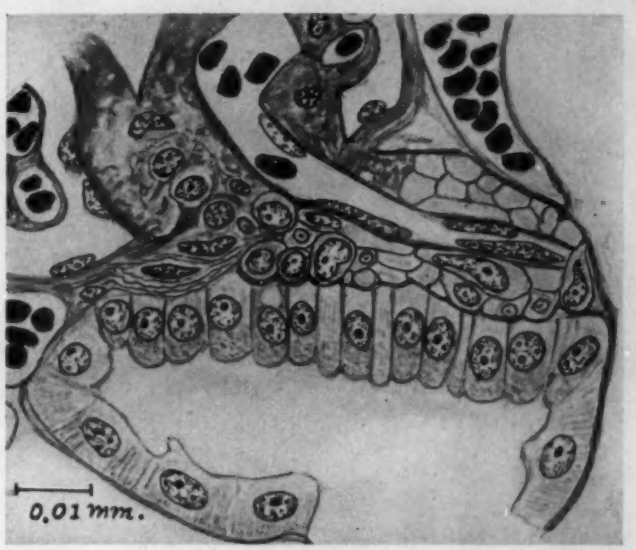

Fig. 7 (normal dog).—Connection of differentiated segment of a distal convoluted segment with the neuromyoarterial apparatus surrounding a vas afferens.

tion, the granulation tissue becomes a scar (dog Thér.; dog Yol.) containing scanty calcium deposits. This nephrosclerosis is still more pronounced in dogs from which the thyroids and parathyroids were removed immediately before calciferol was given (fig. 8). In these animals the renal excretion of calcium is much more increased. Whether these stabilized interstitial scars which reach the renal capsule have a progressive harmful effect on all the glomeruli and tubules is difficult to ascertain.

Steck, Deutsch, Reed and Struck[45] examined the calcium content of the kidney and found as much as 3,464 mg. of calcium per hundred grams of dried

45. Steck, J. E.; Deutsch, H.; Reed, C. J., and Struck, N. H.: Ann. Int. Med. **10**:951, 1937.

tissue after the administration of large doses of vitamin D. Chieffi[46] made similar observations on the kidney of the rat.

It could be suggested that the initial lesion of the tubular epithelium is due to an excess of vitamin D. The parallelism between the extent of lesions and the intensity of calcium excretion is not in favor of this suggestion. Moreover, Coppens and Metz[47] found that the kidney does not easily store vitamin D, in contrast to the liver, the adrenal, the spleen and the lungs.

The spontaneous lymphoid infiltration observed in control dogs (p. 1157) has no relation to the formation of calcium deposits nor does it cause tubular distention. The interstitial process discussed in this part of our paper is not seen

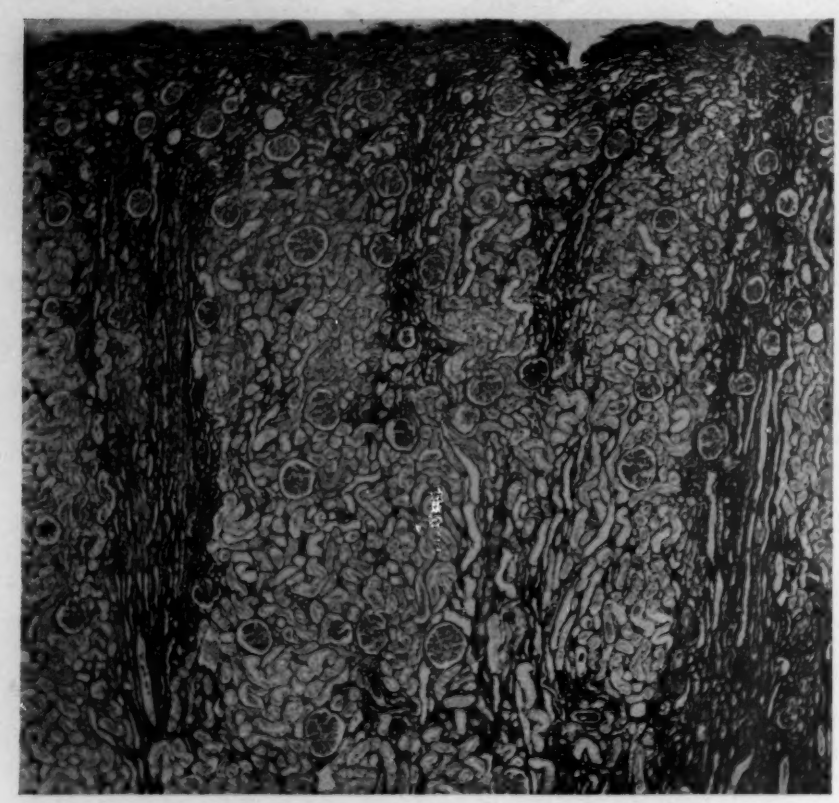

Fig. 8 (dog Les., with thyroid and parathyroids removed; initial dose of calciferol 3 mg. per kilogram, followed by 0.6 mg. per kilogram daily during thirty-seven days; without treatment during forty days).—Scarring of the cortex; atrophy of the cortex corticis. Note the smaller size of the glomeruli in the sclerotic areas, although the arterioles are intact; × 50. The same scarring is seen also to a lesser degree in dog Thér. (table 3).

in control dogs or in animals treated with small or moderate doses of calciferol (tables 1 and 2); it exists in 100 per cent of the dogs given larger doses (table 3).

46. Chieffi, A.: Stud. sassaressi **11**:483, 1933.

47. Coppens, P. A., and Metz, G. A.: Arch. néerl. de physiol. **18**:407, 1933.

The regular incidence of the interstitial process in the series of dogs of table 3 and the parallelism between its extension and the dosage confirms its experimental character.

No relation exists between arteriolar degeneration, on one hand, and tubular or interstitial lesions, on the other. According to the mode of administration of the drug we observe the following combinations: (*a*) no tubular or interstitial damage with severe arteriolar necrosis (dogs 23, 24, 25 and Bas., table 2); (*b*) tubular and interstitial changes without arteriolar necrosis (dogs Guil., Herm. and Yol., table 3); parenchymatous and interstitial changes with arteriolar necrosis (dogs Mad., Ad. and Char., table 3). In other words, severe arteriolar degeneration is independent of increased excretion of calcium.

The stained lipoids of the renal epithelium varied also during our experiments. They disappeared when large doses were given and were restored when the treatment was discontinued. Since the amount of renal lipoids is not constant in the normal dog, a systematic investigation of the lipoid fluctuations does not serve any useful purpose at present.

We will discuss in another paper the significance of the intranuclear crystalloid inclusions so frequently observed in hypervitaminized dogs. They are, no doubt, related to the more amorphic nuclear inclusions seen by Cowdry and Scott [26] in monkeys treated with viosterol.

GLOMERULAR CHANGES

Let us consider dogs 23, 24, 25 and Bas., all showing severe arteriolar lesions. We have already noted the integrity of the myoblastic component of the juxtaglomerular neuromyoarterial apparatus (see p. 1158 and fig. 5 *B*). The nonmyoblastic component of this apparatus enlarges and contains more nuclei than previously (fig. 5 *B*), embedded in a dense fibrillar ground substance. We find also an increase in the size and number of the nuclei present in the wall of the bulblike terminal expansion of the vas afferens. Lipoid infiltration of this area, sometimes observed in normal dogs, is strikingly evident in most of the treated ones. These changes are perhaps the first stage of a particular type of glomerular regression and remind us of some aspects of human glomerulosclerosis. This infiltration does not, however, extend to the glomerular loops.

In spite of severe degeneration of the vasa afferentia, which, however, remain patent, the glomerular tufts are not altered. Also in the D hypervitaminized rabbit a completely calcified vas afferens with enlarged lumen is connected with a normally constructed but congested glomerulus (fig. 9). Perhaps the capillary loops will regress in the long run as the result of a condition of stasis. At any rate, if so,

regression develops tardily, because in a dog under observation for more than a year some of the normal glomeruli were connected with degenerated but patent arterioles.

On the other hand, the glomerular tuft is collapsed when the Henle loops and especially the initial segment of the distal convoluted tubes, in contact with the vascular poles of the glomerulus, are distended by the calcium casts (fig. 10). The glomerular collapse occurs before peritubular infiltration, thickening of the basal membrane and sclerosis set in. The tubular distention is responsible for the glomerular collapse. Let us give our reasons: First of all there is no question

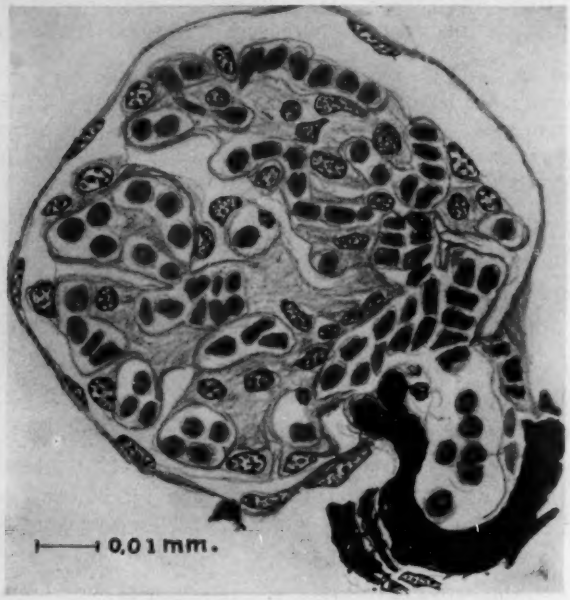

Fig. 9 (rabbit 643).—Patent vas afferens with calcified media connected with a congested glomerulus.

that the excessive excretion of calcium or other noxa damage simultaneously the glomerulus and the tubule, because we have no morphologic evidence of glomerular damage at the onset of the process. The glomerular collapse can be the result only of a lower pressure in the loops, which in turn must be due to a constriction of the vas afferens; when, however, the latter shows completely degenerated walls but remains patent, the glomerular tuft is expanded (fig. 9). This is easy to understand since the arteriolar wall contains no more contractile cells: Vasoconstriction becomes impossible, and there can be no glomerular collapse. On the other hand, it is known that a mechanical irritation of the vas afferens is followed immediately by constriction

(Okkels and Peterfi [23]). Figure 10 illustrates our argument. The distended tubule lying close to the collapsed glomerular tuft must act on the corresponding vas afferens as the needle of the micromanipulator in the experiment of Okkels and Peterfi on the kidney of the frog. Figure 10 illustrates another point: Peritubular granulation tissue reaches the vascular pole of the glomerulus and can also act as an irritating factor on the glomerular arteriole. It stands to reason that this granulation tissue is more abundant at the place where several collecting tubules are grouped (fig. 8). This means that the spastic condition of the arterioles has more chance to be permanent and severe in the vicinity of the collecting tubes. There we find, chiefly, the degen-

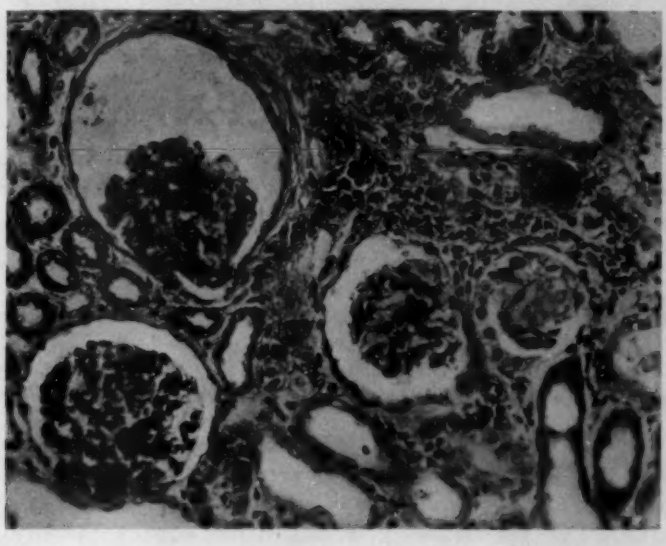

Fig. 10 (dog Guil.).—On the right, a distended convoluted tubule containing a cast and connected with a collapsed glomerular tuft. Intertubular granulation tissue with calcium deposits reaches the vascular pole of one or the other collapsed glomeruli. The collapsed glomerulus in the lower left hand corner is also connected with a distended tubule; × 250.

erated glomeruli in dogs that have been a long time under observation (dog Thér.). Figure 8 illustrates this fact in the thyroparathyroidectomized dog already referred to. The permanent collapse of the glomerular tuft leads to progressive disappearance of the loops, thickening of their axial frame, formation of a sclerotic core surrounded by a syncytial glomerulothel and eventually to a slight cellular reaction of the parietal layer of the Bowman capsule. Cowdry and Scott [26] observed similar lesions in some of their animals. One could suggest also that the glomerulus is affected by the back pressure acting along the

whole extent of the urinary tubule itself. But this view is not in accordance with the facts, since the lumen of tubulus contortus 1 is never or seldom distended.

Sometimes the precipitation of calcium phosphates and the damage of the distal tubules of the nephron are so severe that the interstitial granulation tissue extends over the whole cortex. Then all the glomerular tufts are collapsed. Moreover, their capillaries are blocked by endothelial leukocytes. Not one red corpuscle passes through most of the tufts. Dog Jean (table 3), showing these extensive lesions, died of uremia, although the administration of calciferol had been discontinued for some time. In spite of these severe glomerular lesions, the proximal convoluted segment corresponding to the degenerated glomerulus shows only slight changes of the mitochondria; the nuclei are preserved, and the cell area is not modified.

The pathologic features of these kidneys are satisfactorily understood only if we admit that the distention and degeneration of the least differentiated segments of the nephron are the first to appear and that these changes cause arteriolar spasm which, if protracted, impairs irrevocably the function of the glomerulus.

We agree with Fahr[48] and with most pathologists that in human contracted kidneys the glomerular regression appears under three aspects: (*a*) progressive hyalinization of the tuft, (*b*) progressive capsular proliferation and sclerosis in crescent form, with secondary (?) regression of the tuft, and (*c*) collapse of the capillary loops, followed by a temporary and evanescent cellular reaction of the glomerulothel and massive sclerosis of the tuft, wiping out its architecture.

It is the latter type of regression we encounter in the D hypervitaminized dog; it has no relation to sclerosis of the afferent vessel. Parenchymal and interstitial lesions are, in hypervitaminosis D with hypertension (see the following section of this paper), primary lesions independent of the structural condition of the arterioles but dependent on a disturbance of the general metabolism. These disturbances induce the formation of protein and calcium casts which in turn irritate mechanically the vas afferens and cause glomerular collapse.

PHARMACOLOGIC OBSERVATIONS

In this part of our paper are examined the pharmacologic aspects of the question and the relation between physiologic and morphologic changes. (Further details are given in a paper published elsewhere.[48a])

48. Fahr, T., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1925, vol. 5, pt. 1, p. 156; 1934, vol. 5, pt. 1, p. 807.

48a. Handovsky, H., and Goormaghtigh, N.: Arch. internat. de pharmacodyn. et de thérap. **56**:376, 1937.

Muscular Hypertrophy in Relation to Dosage (table 1).—Hypertrophy of the arteriolar media without regressive lesions (fig. 2 *C, D, E, F and G*) was observed with a daily dose of calciferol ranging from 50 to 720 micrograms per kilogram during a period varying from three to one hundred and fourteen days. Dogs of this series had a normal behavior. This hypertrophy was most marked in dogs 6, 7, 8, 13, 14, 15 and 19. The observation of dog 13 shows that with a daily dose of 720 micrograms per kilogram the hypertrophic effect is reached by the third day. On the other hand, hypertrophy is most striking in dog 19, given much smaller daily doses (110 micrograms per kilogram during one hundred and fourteen days). With a larger dosage (600 micrograms and from 1,500 to 3,000 micrograms per kilogram) or a more protracted treatment, hypertrophic smooth muscle cells are mingled with regressive and even necrotic cells (table 2).

Hypertrophy and Blood Pressure.—Dogs 6, 7, 8, 10, 13, 14, 15 and 19, showing marked generalized visceral arteriolar hypertrophy, at the exclusion of any other regressive lesion, had at the end of the calciferol treatment a high blood pressure, reaching 206 mm. of mercury. The remarkable arteriolar hypertrophy seen in dog 19 (fig. 2 *D*) corresponds to a blood pressure of 200 mm. of mercury, against 135 mm. at the beginning of the experiment. From measurements made at regular intervals on two trained animals (dogs 23 and 19), it appears that the curve of the blood pressure rises as early as during the first days and becomes stabilized at a level of 200 mm. of mercury later on. When the treatment with calciferol was discontinued, the blood pressure gradually returned to normal (dog Bas.) (Handovsky [41]). When arteriolar hypertrophy was less marked as the result of a lower dosage (from 50 to 70 micrograms per kilogram), no rise of the blood pressure was registered except in dogs 4, 21 and 22, receiving the daily dose of 50 micrograms per kilogram for about a hundred days. Consequently, a certain degree of parallelism exists between the intensity of arteriolar hypertrophy and the blood pressure.

However, it must be emphasized that concomitance of atrophic and even necrotic cells in the arteriolar media of the kidney is not incompatible with high blood pressure. Dogs 23 and 24 had at the end of the experiment an arterial blood pressure amounting to 206 mm. of mercury. Yet in the kidneys of these animals regressive arteriolar changes were present (dog 23). They were observed in the kidneys and spleen of dog 24, but the remainder of the arteriolar system was decidedly hypertrophic or normal. Moreover, even in these damaged kidneys, arteriolar segments were still intact and the afibrillar muscle cells preserved (fig. 5 *B* and *E*).

Appelrot's [49] observations also show the hypertensive effect of vitamin D. With a daily dosage of irradiated ergosterol (vigantol) ranging from 70 to 90 micrograms of vitamin D per kilogram during a period of from fifteen to twenty-five days a rise of the blood pressure averaging 36.6 mm. of mercury was registered. Appelrot's experimental conditions cannot be compared accurately with ours.

One of us (Handovsky [41]) discussed in a previous paper the eventual cause of the hypertensive effect. Four factors, at least, come under consideration: (*a*) the thyrotropic effect of calciferol (vitamin D_2) observed by us [50] previously and confirmed since by measurements of the basal metabolism (Deutsch, Reed and Struck [51]); (*b*) the increased tone of the hypertrophic muscle cell; (*c*) the increased sensitivity of the arteriolar wall to epinephrine; (*d*) a central excitation. If the hypertensive effect has a central origin, the hypertrophy of the arteriolar media and particularly of the afibrillar leiomyoblasts forming the link between nerves and contractile cells should contribute to the full efficiency of the tonifying nervous impulse. It must be emphasized that with subacute lethal doses (from 13 to 20 mg. per kilogram) the arteriolar necrosis is generalized and the blood pressure becomes very low. In this case, the arterioles have wide, irregular lumens.

Arteriolar Hypertrophy and Sensitivity to Epinephrine.—As suggested, the hypertensive effect of vitamin D_2 may depend partly on increased sensitivity of the arterioles to epinephrine. The hypertrophic arterioles of dogs 10, 11, 7, 8, 14, 15, 19, 12 and 13 responded with constriction to extremely small doses of epinephrine (table 1).

Cannon and Lyman [52] found that in normal dogs the vascular response was obtained with a minimal dose of 1 microgram per kilogram; when the animals were anesthetized with chloralose (chloral hydrate and dextrose) we observed exceptionally a response with 0.1 microgram per kilogram. In the dogs of table 1 the minimal effective dose varied from 0.0005 to 0.1 microgram per kilogram. All these dogs showed a polymorphic adrenal medulla, pointing to an increased output of epinephrine. On the other hand, the thyroids of these animals were overactive,[50] and on the evidence of Asher [53] and of Cannon and Smith [54] it is possible that sensitization of the sympathetic nervous system by the thyroid hormone accounts for the increased sensitivity of the arterioles to epinephrine. We know also that thyroid secretion interferes with the chemical composition of the blood, the slightest change of which influences the arteriolar sensitivity.

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49. Appelrot, S.: Am. J. Physiol. **105**:294, 1933.

50. Goormaghtigh, N., and Handovsky, H.: Compt. rend. Soc. de biol. **118**: 1616, 1935.

51. Deutsch, H.; Reed, C. J., and Struck, H. C.: Am. J. Physiol. **117**:1, 1936.

52. Cannon, W. B., and Lyman, H.: Am. J. Physiol. **31**:376, 1913.

53. Asher, L., in Hirsch, M.: Handbuch der inneren Sekretion, Leipzig, Curt Kabitzsch, 1928, vol. 2, p. 192.

54. Cannon, W. B., and Smith, A. S.: Am. J. Physiol. **60**:476, 1922.

Inverse Effect of Epinephrine on Blood Pressure and Incipient Hypertrophy of the Arterioles.—It is rather remarkable that the exquisite sensitivity referred to was replaced by an inverse reaction to epinephrine in dogs 1, 2, 3, 4, 21 and 22 (table 1), receiving smaller doses of vitamin D (from 40 to 70 micrograms per kilogram) and showing only incipient hypertrophy but definite morphologic signs of increased cell metabolism (fuchsinophil bodies and vacuoles). Doses of epinephrine of from 0.05 to 0.2 micrograms per kilogram given intravenously to dogs anesthetized with chloralose were followed by a marked drop of the blood pressure. The intensity of this inverted reaction decreased after the subsequent injections.

Cannon and Lyman [52] found that small doses of epinephrine have a hypotensive effect in carnivores. Yet we never observed this inverted reaction in hundreds of dogs narcotized with chloralose in this pharmacologic institute.

The exact significance of this inverted reaction escapes our knowledge. It would be interesting to test the sensitivity of the arterioles to epinephrine at regular intervals in order to make out whether the cell hypertrophy of the media, corresponding to increased sensitivity to epinephrine, is preceded by a transitional stage in which the smooth muscle cells relax under the influence of small doses of epinephrine.

Dosage of Calciferol in Relation to Arteriolar and Renal Pathology.—From a pathologic and pharmacologic point of view, a distinction must be made between the daily administration of calciferol and the administration of the drug at regular intervals.

By daily doses of from 500 to 600 micrograms per kilogram we obtained, from the fourteenth day onward, shrinkage of muscular cells in interlobular prearterioles and glomerular arterioles and a discrete necrotic process in the interlobular prearterioles (dogs 20, 23, 24, 25 and Bas., table 2). These regressive signs became marked in dogs treated over periods of from seventeen to forty-five days. In this series the tubules and interstitial tissue were unchanged; the necrotic arterioles had wide lumens; the endothelium was intact; there was no thrombosis.

On the other hand, when calciferol was administered at regular intervals varying from two to five days, much larger doses were needed to damage the arterioles (dogs Guil., Herm., Ad., Mad., table 3). Three of these dogs (Herm., Ad. and Mad.) can be compared with dogs 24, 23 and 25 of table 2 in respect of the duration of treatment. The arteriolar lesions obtained by continuous daily administration were much more pronounced. Dogs surviving the spaced administration of large doses of calciferol showed less severe arteriolar lesions, because the atrophic or shrunken cells had a chance to recover. Only the necrotic cells remained.

Dog Yol. (3,000 micrograms per kilogram nine times; table 3), surviving the calciferol treatment fifty-six days, was practically free from arteriolar lesions; this proves that no necrotic cells were present at the end of the calciferol feeding. Dogs Char. and Jean (3,500 micrograms per kilogram eleven times), surviving, respectively, twenty-eight and forty-eight days, showed discrete arteriolar lesions. Dog Thér. (300 micrograms per kilogram nine times during twenty-two days) survived four hundred and twelve days; numerous necrotic cells were still present in the media. However, their number was inferior to that encountered in dogs 23 and 24 (killed after forty-five days' continuous treatment) and in dog Bas., given an initial dose of 3,000 micrograms per kilogram, followed by daily doses of 600 micrograms per kilogram during thirty-two days, and surviving one hundred and nineteen days.

Although the arterioles are less injured by spaced large doses (from 1,500 micrograms per kilogram), this mode of treatment with calciferol is very harmful: Important regressive changes occurred in the liver (endothelial lesions), spleen (toxic lesions in the malpighian bodies) and especially the kidneys. As was noticed already by Demole and Fromherz,[55] hypercalcemia reached a high level (from 15.3 to 23.6 mg. per hundred cubic centimeters). It caused severe excretion of calcium, with subsequent tubular, interstitial and glomerular lesions. When this mode of treatment was discontinued in due time, these lesions led to the formation of scars in the kidney and the degeneration of a number of glomeruli. When the calciferol feeding lasted too long (dogs Jean and Mad.) the animal died with uremic symptoms.

From a pharmacologic point of view, it was also necessary to determine the minimum single dose causing arteriolar lesions. While still having a hypertrophic effect on most of the smooth muscle cells, a single dose of 6,000 micrograms per kilogram causes a marked shrinkage of many muscular cells of the interlobular prearteriole. In dog Gab., with a dose of 7,000 micrograms per kilogram, distinct regressive changes occurred in the prearterioles of the neurohypophysis. Here again we come across the special sensitivity to vitamin D of a vascular segment placed between the arteries and the precapillary arterioles. In this series, no definite parenchymal renal lesions were observed except in dog Louis, with a dose of 9,000 micrograms per kilogram, showing a few hyaline and calcium casts, a discrete tubular distention and glomerular retraction. (See also Handovsky and Goormaghtigh.[48a])

We determined also the single subacute lethal dose. Four adult dogs were given, respectively, 13,000, 15,800, 16,700 and 20,000 micrograms per kilogram. They died, respectively, after one hundred and thirty, seventy-four, one hundred and forty and one hundred and thirty hours. Massive arteriolar necrosis was found in most of the organs except

55. Demole, V., and Fromherz, K.: Arch. f. exper. Path. u. Pharmakol. **146**: 347, 1929.

the liver, where the regressive lesions had attained only the stage of clear tumefaction. Calcium casts in the kidney were abundant, and tubular distention was marked.

Clinical Behavior in Relation to Dosage and Arteriolar Lesions.—We shall summarize our observations: (*a*) Arteriolar necrosis or regression in the kidney and sometimes in the spleen, without renal parenchymal or glomerular lesions (dogs 23, 24 and 25, table 2; 600 micrograms per kilogram daily). This finding corresponds to a normal clinical behavior. Blood pressure is high. Schiff,[55] working on the rabbit, was also struck by the lack of parallelism between the extent of the vascular lesions and the development of morbid symptoms.

(*b*) Arteriolar regression (or necrosis) with renal parenchymal and progressive glomerular lesions (dose from 1,500 to 3,500 micrograms per kilogram at regular intervals). With these doses there are individual variations, but on the whole the clinical symptoms depend on the duration of the treatment. After the fourth dose, the animals become shy and moody; after the seventh, they are very ill. We did, however, give eleven doses to two dogs without any immediate fatal result. Generally, after the seventh dose, the animals feel nauseated and vomit; still, they eat a little food; they lose weight (as much as 38 per cent); the bilious sickness increases; diarrhea, anorexia and asthenia set in; the blood pressure is low; hypercalcemia is marked (table 3, from 16 to 23 mg. per hundred cubic centimeters); the blood urea attains gradually a high level (122 mg. per hundred cubic centimeters), an observation in agreement with those of Spies and Glover [56] on the rabbit. The blood is unusually viscous; the taking of blood samples for analysis proves difficult and aggravates the general condition. This explains the incompleteness of our data on the chemical composition of the blood: We did not want to interfere too much with the animals in order to get comparable morphologic results. Cowdry and Scott [26] discussed the disturbing influence of frequent taking of blood. No doubt, the progressive uremia accounts for most of the symptoms. Whatever the shortcomings of our clinical study, we want to emphasize that this severe accompaniment of symptoms corresponds, to a great extent, to that observed by Collip and others [57] in dogs overdosed with parathyroid extract. About the similarities and dissimilarities of the mechanisms of the action of vitamin D and parathyroid extract, we refer to McJunkin, Tweedy and McNamara's [58] recent paper.

Observations in Growing Dogs.—Two fifty-six day old puppies of a litter of four were given every other day 600 micrograms of calciferol per kilogram. One

56. Spies, T. D., and Glover, E. C.: Am. J. Path. **6**:485, 1930.

57. Collip, J. B.; Clark, E. P., and Scott, J. W.: J. Biol. Chem. **63**:439, 1925.

58. McJunkin, F. A.; Tweedy, W. R., and McNamara, E. W.: Am. J. Path. **13**:325, 1937.

was killed with its control after fifty days; the other pair, one hundred and seven days after the beginning of the experiment. No vascular lesions were observed, but calciferol arrested growth (thymus regression). The arterioles of growing animals seem more resistant to the damaging effect of the drug. (See also Handovsky and Goormaghtigh.[48a])

COMMENT

Pathologic Observations.—When the arterioles remain patent, the degeneration of their walls caused by vitmain D has no unfavorable effect on the glomerulus except for the congestion and expansion of its loops; the same conclusion can be drawn from a careful study of the human contracted kidney and from a perusal of the illustrations of the papers of Russell,[27] Schuermann and MacMahon [40] and Klemperer and Otani.[59] The importance of vascular damage in relation to glomerular regression has been overstressed.

On the other hand, less damaged, normal or even hypertrophic arterioles were often encountered with collapsed or degenerated glomeruli. Consequently, except in the case of obliteration or marked narrowing of the lumen, the arteriolar lesions are not responsible for the glomerular regression. What is then the controlling factor? We have suggested that in hypervitaminosis D_2 it is the protracted spastic condition of the afferent arteriole induced not by the noxious agent directly, as Volhard [60] admitted in regard to hypertensive nephritis, but by the distention of the distal part of the nephron or by the irritative effect of the subsequent peritubular inflammation. This is not mere speculation. Our assertion is based on unquestionable facts: (*a*) the constrictive response of the vas afferens to mechanical interference (Okkels and Peterfi [23]); (*b*) the constant anatomic connection between the vascular pole of the glomerulus and the distal part of the distal convoluted tubule, making a mechanical irritation of the vas afferens unavoidable; (*c*) the coincidence of tubular distention or of peritubular inflammation with glomerular collapse or regression.

In other words, the glomerular vascularization is conditioned for a great part by the tubular status. This conclusion is of great value in the morphologic study of the contracted kidney. The significance of tubular lesions in nephrosclerosis has never been correctly understood. Most authors admit that their regression follows that of the glomerulus. Yet in the light of our observations Aschoff's "inaction atrophy" proves to be a speculative view: Often the degenerated glomerulus is connected with a normal proximal convoluted segment; the latter is even hypertrophic in chronic Bright's disease, as Oliver and Lund [61] have

59. Klemperer, P., and Otani, S.: Arch. Path. **11**:60, 1931.

60. Volhard, F.: Die doppelseitigen hämatogenen Nierenerkrankungen, in von Bergmann, G., and Staehlin, R.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1931.

61. Oliver, J., and Lund, E. M.: Arch. Path. **15**:755, 1933.

proved by reconstructing the nephron. Neither is a secondary tubular regression satisfactorily explained by a reduction of the blood supply (Fahr): As long as the blood can be diverted along vascular shunts, the sclerosis of the glomerular tuft cannot modify in any important way the behavior of the capillaries and the vascularization of the tubules. Oliver and Luey[12] found that these shunts become conspicuous in human contracted kidneys; this question, however, deserves new investigation. In our hypervitaminized dogs tubular lesions occurred before any changes of the capillary net.

Tubular lesions in chronic Bright's disease have only recently received the attention they deserve in the remarkable work of Oliver and his associates.[62] The modifications of the tubular architecture are comparable to those encountered in dogs surviving the calciferol treatment a long time. Yet this analogy is obtained in spite of the fact that we never succeeded in reducing the lumens of the arterioles by lesions of their walls. The significance of the observations of Oliver and collaborators becomes clear when our observations and deductions about the functional relation between glomerulus and tubule are taken into account.

That the vascular lesions alone are not responsible for the parenchymal changes in the contracted kidney can be deduced indirectly from the work of Kimmelstiel,[63] who devoted much attention to an ascending process of tubular regression. Oberling[64] considered also the possibility of the concurrence of "an ascending nephritis" with vascular sclerosis. Finally, the distribution of the sclerotic patches in the contracted kidney receives its correct explanation if we admit that in chronic Bright's disease, as in D hypervitaminosis, the tubular lesion is independent of a primary lesion of the glomerulus.

Tubular Changes.—It must be borne in mind that the tubular lesions seen in hypervitaminosis D_2 are the result of profound changes in the chemical make-up of the blood. When hypercalcemia and the excretion of calcium are slight, arteriolar necrosis occurs without parenchymal or glomerular lesions. No sign of renal insufficiency is evident. On the other hand, when, as the result of larger doses, the hypercalcemia and the excretion of calcium are marked, we find a combination of arteriolonecrosis and nephritis (glomerular, tubular and interstitial lesions) and observe a distinct tendency toward uremia.

These two contrasting histologic conditions remind us of the distinction made between benign and malignant nephrosclerosis. On the

62. Oliver, J., and Luey, A. S.: Arch. Path. **19**:1, 1935. Oliver and Luey.[12] Oliver and Lund.[61]

63. Kimmelstiel, P.: Am. J. Path. **11**:483, 1935.

64. Oberling, C.: Ann. d'anat. path. **1**:217, 1924.

evidence accumulated in this work, it seems that both diseases could be caused by the same etiologic factor: In the first case, the noxious agent, being at a low concentration, would cause mild clinical manifestations; in the second, the same agent at a higher concentration, would be responsible for arterial necrosis and profound disturbances of the general metabolism, resulting in severe renal lesions (malignant nephrosclerosis). Our results give thus some support to Volhard [60] in his controversy with Fahr,[48] who admitted the superimposition of an exogenous factor in malignant nephrosclerosis.

However, a definite conclusion in this respect cannot be reached as we produce with vitamin D only exceptionally intimal reactions of the arterioles comparable with those of malignant nephrosclerosis.

In the meantime, let us consider the significance of tubular lesions from a general point of view. These lesions are of primary importance in the development of renal insufficiency. They are the result of metabolic changes and severe alterations of the chemical nature of the blood. Consequently, a closer study of the excretion of metabolites will give a clearer insight into the clinical and pathologic aspects of hypertensive diseases.

Pharmacologic Observations.—Our systematic investigations prove that in normal dogs two types of lesions must be avoided when one is administering vitamin D with a therapeutic purpose: (*a*) the arteriolar and prearteriolar necrosis; (*b*) the tubular lesions. The latter are the most dangerous, because they lead to uremia.

Our pharmacologic investigations establish that doses of about 100 micrograms per kilogram given daily during a period of a hundred days are not only harmless but helpful: They keep the arterial system in perfect condition; they lead to hypertrophy of the arteriolar wall, an effect which might be beneficial in a patient with arterial hypotension. This condition must be avoided, of course, in patients suffering from any hypertensive disease. At this dosage, moreover, vitamin D stimulates the thyroid [65] and the liver cells and keeps the animal fit.

We have stated that daily doses of from 600 micrograms per kilogram up cause regressive lesions in arterioles, leading eventually to cell necrosis (in from seventeen to forty-five days). It is evident that these doses cannot be recommended for therapeutic use. If, however, in this respect we should have overstepped the mark, it is not sure that we have done irremediable harm. As long as the necrotic stage has not been reached, the smooth muscle cell can recover; moreover, only the kidney and, in a smaller measure, the spleen have damaged arterioles with this

65. Handovsky and Goormaghtigh.[48a] Goormaghtigh and Handovsky.[50] Reed and others.[51]

dosage. Even there, the lesions show a segmentary distribution. The arteriolar lumen remains patent, and the renal parenchyma is unchanged. The blood pressure remains high, the hypercalcemia is moderate, and the animal keeps in good condition. What will become of it after two or three years' observation cannot be said. If the calciferol feeding has not lasted more than one hundred days, it seems probable that no marked ultimate ill effect will be registered.

These conclusions refer to adult animals. Arterioles of puppies or young dogs resist better an overdosage of vitamin D, but here we are confronted with another harmful effect: Doses of 600 micrograms per kilogram given every other day cause thymus regression and retard growth.

Another important remark must be made: This discussion of dosage holds good only as long as we are dealing, from the onset of the calciferol treatment, with normal dogs. When, for instance, the thyroid has been removed or when thyroid aplasia or marked hypothyroidism are present, the aspect of the problem changes entirely and the indicated doses cause gross lesions of the aorta. We have already reported these facts [50] but intend to give a detailed account of them later. (See also Handovsky and Goormaghtigh,[48a] and Handovsky.[66])

The regular determination of the blood calcium seems to be the most reliable way to follow the effects of the treatment. A value of 13 mg. per hundred cubic centimeters should be cause for alarm. It is the prelude to massive calcium excretion, tubular distention and subsequent glomerular regression.

SUMMARY

Calciferol (vitamin D_2) in moderate daily doses caused hypertrophy and morphologic changes indicative of increased cell metabolism in both types of cells in the arteriolar media (the ordinary smooth muscle cell and the afibrillar cell). Larger but nonlethal doses do not affect the afibrillar cells but cause regressive changes in the smooth muscle cells. Some of these changes are reversible after discontinuation of the treatment. The renal interlobular prearteriole is more sensitive to the effect of calciferol than the arteriole. These regressive changes are present to a smaller extent in the arterioles of the spleen, neurohypophysis, thyroid, gonads, adrenal and pancreas. In the normal dog calciferol leaves the aorta macroscopically unchanged. With the dosage employed, alterations of the elastic membrane or intimal reactions are rarely observed. Arteriolar calcinosis is absent. Calciferol in heavy doses causes necrosis of the arteriolar media, a lesion similar to that found in scarlet fever and eclampsia.

66. Handovsky, H.: Schweiz. med. Wchnschr. **68**:425, 1938.

In the dog's kidney calciferol causes arteriolonecrosis, with or without nephritis, depending on the dose employed. An explanation of the pathogenesis of the renal lesions is offered.

Doses of from 50 to 70 micrograms per kilogram cause inversion of the vascular response to epinephrine. Heavier doses increase the sensitivity of the hypertensive response.

Arterial hypertension develops when the treatment is maintained at a dosage of from 100 to 700 micrograms per kilogram per day. Doses not exceeding 250 micrograms per kilogram have a thyrotropic effect.

The significance of these observations in the problem of human arteriosclerosis is discussed.

HYDROLYSIS OF ESTERS IN THE INTERCELLULAR MEDIUM

AN EXPERIMENTAL STUDY

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The artificial introduction into the tissue spaces of materials which can be conveniently manipulated in the laboratory and the study not only of the reaction of the tissues to these materials but also of the reaction of the materials to the imposed environment constitute one method of approach to the mechanisms which are operative in the intercellular phase. The results of experimental studies which have been reported indicate that there is a difference in the behavior of glyceryl and methyl esters of fatty acids during the residence of these materials in the intercellular environment of the subcutaneous tissues.[1] This study is concerned with an investigation of the hydrolysis of various esters of fatty acids in the intercellular medium and with the influence of the products of that hydrolysis on the tissues.

MATERIALS AND METHODS

A partial homologous series of normal saturated acids was purified to satisfy the following physical constants:

	Melting Point, C.
Capric acid	31 –32
Undecylic acid	22.5–23.5
Lauric acid	42 –43
Tridecylic acid	39.5–40.5
Myristic acid	52 –53
Palmitic acid	61 –62
Margaric acid	58 –59
Stearic acid	69 –70

The methyl esters of these acids were prepared by the use of methyl sulfate and isolated by distillation in a vacuum.

Higher alcohols esterified with saturated acids had the following constants:

	Melting Point, C.	Boiling Point, C.
Ethyl stearate	30–32	.....
N-butyl stearate	18–20	.....
Isoamyl stearate	.....	185–190 (1 mm.)
Secoctyl stearate	.....	190–194 (1 mm.)
Tetrahydrofurfuryl palmitate	.....	195–198 (1.5 mm.)

From the Department of Pathology, Harvard Medical School.

1. Hass, G. M.: Arch. Path. **26**:956, 1938.

Several unsaturated acids and their esters were prepared as follows:

Oleic acid was obtained by hydrolysis of olive oil. It was separated from the saturated acids by the lead salt–ether method and was purified by repeated distillation of the methyl ester in high vacuum. Also a second product, designated as oleic acid, chemically pure, free from linoleic acid, was available. Both products were converted to methyl esters. Other esters of oleic acid employed in the experiments were the following:

	Boiling Point, C.
Ethyl oleate	205–208 (10 mm.)
N-butyl oleate	180-195 (2 mm.)

Elaidic acid was obtained by subjecting oleic acid to the action of nitrous acid. The high melting isomer was separated as the ether-insoluble lead salt and after conversion to the acid was purified by recrystallization to a melting point of 45-47 C. and by distillation of the methyl ester.

Erucic acid (melting point, 31-33 C.) was obtained as a commercial product and converted to the methyl ester.

Ricinoleic acid was obtained by converting sodium ricinoleate (practical) to the methyl ester. A second fractional distillation yielded a product (boiling point, 180-182 C. at about 1 mm.) which gave on hydrolysis a fairly pure ricinoleic acid (melting point, 5-8 C.).

Chaulmoogric acid was isolated from a technical product by recrystallization to a melting point of 66-68 C. and was purified by vacuum distillation of the methyl ester (boiling point 170-172 C. at about 1 mm.). Ethyl chaulmoograte (boiling point, 210-222 C. at 20 mm.) also was utilized.

Linoleic and linolenic acids were separated from the products of hydrolysis of linseed oil. After partial isolation of the unsaturated acids by the lead salt–ether method, the acids were brominated directly in cold acetic acid. The hexabromides, insoluble in ether, were purified by recrystallization to a melting point of 176-178 C. The ether-soluble tetrabromides were largely separated from the dibromides by use of purified petroleum benzene (petroleum ether). The linoleic and the linolenic acids were regenerated from their respective bromides with zinc dust. After conversion to the methyl esters they were purified by vacuum distillation. The linolenic acid so obtained, according to Erdmann and Bedford,[2] is composed of a beta isomeric form as well as the alpha natural form of the acid.

Several glycerides were utilized in the experiments. Some were commercial products, and others were prepared in the laboratory by the interaction of glycerol and acyl chlorides in cold quinoline. Oxalyl chloride was used in the preparation of the halides which were isolated for the preparation of the glycerides. The saturated glycerides were:

	Melting Point, C.	Boiling Point, C.
Tristearin	70– 72	.....
Tripalmitin	64– 66	.....
Tricaproin	.....	225–228 (13 mm.)
Triacetin	.....	152–154 (22 mm.)

Triolein, an oil, was the only unsaturated glyceride of known composition which was employed. Mixed glyceryl esters of unsaturated acids derived from cod liver oil were synthesized. Their constitution was not determined.

2. Erdmann, E., and Bedford, F.: Ber. d. deutsch. chem. Gesellsch. **42**:1324, 1909.

Olive oil, cod liver oil and linseed oil from which the free acids were removed prior to injection served as a source of mixed naturally occurring saturated and unsaturated glycerides of widely different compositions.

All materials were introduced subcutaneously into the quadrants of the abdominal wall in young guinea pigs (from 250 to 350 Gm.). The liquid compounds were injected with a 1 cc. graduated tuberculin syringe in 0.1 cc. doses. The solid compounds, tristearin and tripalmitin, were injected in doses of from 10 to 20 mg. suspended in Tyrode's solution.

Records of the progress of the lesions were made every three or four days. At the end of two weeks, the animals were put to death, and segments of the abdominal wall of each were fixed for from twenty-four to forty-eight hours in solution of formaldehyde U.S.P. (1:10). The segments were then cut in slices from 2 to 3 mm. thick. Blocks including the site of the reaction were dehydrated in graded alcohols and embedded in paraffin. The sections for microscopic study were stained with hematoxylin and eosin.

Methods for Estimating the Hydrolysis of Esters in the Intercellular Medium.—Three general histologic methods were employed for evaluating the hydrolysis of liquid esters in the intercellular medium under the conditions of these experiments.

The first method depends on the detection of some product of hydrolysis or some compound derived from one or more products of hydrolysis of the ester. This method is most useful when a liquid ester yields on hydrolysis some stable compound which is relatively insoluble in the intercellular medium and solid at the temperature of the tissues. In general, it should make little difference whether the alcohol or the acid fulfils this requirement. It is not essential that the product or products be crystalline, although in these experiments many of the solid insoluble products were crystalline rather than amorphous. In either instance, the encapsulation of the solid, relatively insoluble products of hydrolysis by tissue cells, especially giant cells and macrophages, aids materially in establishing the fact of hydrolysis in vivo. Under appropriate conditions this method is very satisfactory and enables one to state whether or not hydrolytic cleavage of ester linkages has occurred.

The delicacy of the method depends on several factors, among which the most important seem to be: first, the local intercellular hydrogen ion concentration; second, the extent to which the ester is hydrolyzed; third, the melting point of the products of hydrolysis, and fourth, their stability, their crystallizing power and their solubility in the medium of the tissues and the ester substrate. The normal hydrogen ion concentration of the tissues in the intercellular phase favors the formation of acids rather than of salts of acids as primary products. The importance of the influence of the second factor varies with the composition of the ester. Under ideal conditions, such as those in the zones of reaction to ethyl and n-butyl stearate, small quantities of products of hydrolysis are easily detected in the presence of a large amount of residual ester. The third factor, namely, the melting point of any given product is of primary importance. The melting point must be above the temperature of the tissues in which the product is formed, if the material is to stimulate the classic tissue reactions to solid foreign materials. It is not necessary that the solid product have either a melting point or a crystal form. The reaction of the tissues in general may be depended on to differentiate the solid substance from the liquid ester. If a product is semisolid or simply of greater viscosity than the ester, the utility of the method for evaluating hydrolysis is questionbale. The fourth factor, namely, the

stability of products of ester hydrolysis, their crystallizing power and their solubilities in the medium of the tissues and the ester substrate, represents a number of variables. So far as the saturated esters employed in these experiments are concerned, the variables are of little importance. However, in dealing with certain types of unsaturated esters, the instability of the esters and products of hydrolysis in the intercellular phase is a factor of importance and greatly influences the nature of the tissue reactions. This particular aspect of the analysis will not be considered in this report.

The second method by which hydrolysis of an ester may be detected under the conditions of these experiments is based on differences in solubilities of esters as compared with products of hydrolysis. Most of the liquid esters employed in these experiments have a low solubility in the intercellular medium, while the liquid products of hydrolysis or major compounds derived from these products have a much higher solubility. The amount of encapsulated residual material, usually in the form of globules, aids materially in these instances in estimating the degree of hydrolysis. The method is most dependable when the tissue reaction is mild or proliferative, so that encapsulated globules or residual ester may be conveniently detected and often differentiated from the residual liquid products of hydrolysis by cooling the tissues to appropriate temperatures before and during fixation in the solution of formaldehyde.

The third method for estimating hydrolysis of an ester depends on the difference between the character of the inflammatory reaction as stimulated by the ester and the reaction excited by the products of hydrolysis. This method is useful because experiments show that esters composed of stable long chain acids and simple alcohols excite an intense inflammatory reaction not by virtue of their composition as esters but rather by virtue of the products of their hydrolysis. This method is limited in dealing with esters which are unstable or are composed of unstable compounds, such as the esters of highly unsaturated acids. In such instances the tissue reactions are not understandable by considering only the effects of products of hydrolysis.

EXPERIMENTAL RESULTS

An interpretation of the experimental data depends in part on a knowledge of the changes occurring in the compounds during their residence in the tissues. Hydrolysis of esters with the primary formation of acids and alcohols will be considered here. Other questions, such as those relating to oxidation, formation of salts of acids, isomerization of certain compounds or synthesis in situ of elementary components into more complex substances which may be identified, will be considered elsewhere.

Hydrolysis of Methyl Esters of Saturated Acids.—The experimental results offer ample proof that the methyl esters of long chain saturated acids (C_{12} to C_{18}) are hydrolyzed during their residence in the intercellular phase. The intensity of the inflammatory reaction at the sites of injection of these esters is of a high order. There are few encapsulated globules of unhydrolyzed ester. The hydrolysis proceeds fairly rapidly and to completion more quickly with the esters of short chain than with those of long chain acids. The principal detectable product

is in the form of an acid which has crystallized out in the zone of reaction. The pattern of the crystals at the boundary of the granulation tissue often resembles an arrangement which is common in the crystallization at surfaces in vitro. The long axes of the crystals in such instances are normal to the boundary. The tissue cells and their processes conform to this arrangement. Long processes of cells extend for great distances along the surfaces of the oriented crystals, thereby offering a resemblance to the pattern of cell processes in some normal structures and in several pathologic lesions of diverse etiology. In other terms, the polarization of fibroblasts, histiocytes, macrophages and giant cells is determined in this instance by purely physical conditions. As one descends the series of methyl esters of saturated acids, this type of polarized reaction stops abruptly. There is an exact correlation between the type of reaction and the melting point of the acid products of hydrolysis. Tridecylic ester (liquid) yielding tridecylic acid (melting point, 39.5-40.5 C.) and lauric ester (liquid) yielding lauric acid (melting point, 42-43 C.) are the lowest members of the series of esters to excite this response. Undecylic ester and capric ester yield, respectively, undecylic acid (melting point, 22.5-23.5 C.) and capric acid (melting point, 31-32 C.). These acids are liquid at the temperature of the body, and the polarized cellular reactions to crystalline deposits are absent. For this reason the conclusion that the methyl esters of capric and undecylic acids are rapidly hydrolyzed in the same manner as the esters of higher homologous acids is reached by application of the second and third methods of estimating hydrolysis. There is an intense inflammatory response, and much of the injected ester in each instance has disappeared from the zone of reaction. The conclusion is reenforced by the induction of crystallization of the residual liquid acids by the cooling of the tissues before and during fixation in the solution of formaldehyde. The acids crystallize out in the exudate. The exudate solidifies during fixation in the solution of formaldehyde. The crystals are soluble in the solvents used in preparation of the sections, but casts of the crystal forms remain, and they are easily recognized as clefts in the exudate as studied in paraffin sections.

The experimental results give no dependable means for differentiating natural from unnatural acids or esters. The rate of hydrolysis of the esters of comparable natural and unnatural acids is about the same. Furthermore, the natural and unnatural acids which are liberated by hydrolysis stimulate similar tissue reactions.

Hydrolysis of Methyl Esters of Unsaturated Acids.—The readiness with which hydrolysis of the unsaturated esters may be detected by the three methods which have been presented depends primarily on the nature of the acyl group of the ester. The stability and melting point of elaidic acid permit a satisfactory conclusion to be reached in the

instance of methyl elaidate. The crystalline deposits and tissue reactions to crystalline deposits of elaidic acid are conspicuous in the regions of injection of the liquid ester. Furthermore, there are no residual globules of encysted ester, and the inflammatory reaction is severe. A similar response was expected in the region of injection of methyl chaulmoograte inasmuch as chaulmoogric acid has a melting point of 66-68 C. Contrary to prediction, no crystal clefts or tissue reactions to crystals are found. The inflammatory reaction is moderately severe, and a few globules of encysted material remain in the intercellular phase. These findings are acceptable as satisfactory evidence of hydrolysis, but no explanation is offered for the absence of crystal deposits. It may be that the compound is degraded in situ. At the site of injection of the methyl ester of erucic acid, there is a severe inflammatory reaction. Several globules of encapsulated liquid material remain in the intercellular phase. This material solidifies on cooling and melts at 25-27 C. This is good evidence that the liquid material is principally erucic acid (melting point, 30-31 C.).

The methyl esters which yield on hydrolysis liquid unsaturated acids that are not readily crystallizable in situ by the methods employed (oleic, ricinoleic, linoleic and linolenic acids) require a more careful consideration. It is clear that much of the material injected as an ester in each instance disappears from the intercellular phase. Furthermore, the magnitude of the inflammatory reaction in the region of injection of each ester approaches that which is characteristic of the corresponding acid. The instability of highly unsaturated esters in the intercellular medium, and some findings which are at variance with predicted results indicate that products other than those derived from hydrolysis of these esters are formed in the zone of reaction. The influence of these products on the tissues will be considered elsewhere. The sum total of evidence, however, permits the general conclusion that methyl esters of the unsaturated acids are hydrolyzed at a rate comparable to that of the methyl esters of saturated acids.

Hydrolysis of Higher Primary and Secondary Alcohol Esters.—In order to strengthen the conclusions as obtained in the study of methyl esters and to test the methods still further, a study of higher alcohols esterfied with standard saturated and unsaturated acids was made.

The ethyl, n-butyl, isoamyl and secoctyl esters of stearic acid excite a response on the part of the tissues that is different from that observed with methyl stearate. The ethyl ester stimulates a mild reaction. A large quantity of the liquid ester remains at the site of injection in the form of encapsulated globules, which are easily detected in the sections. Deposits of crystals and cellular reaction to the presence of crystals are not conspicuous. This evidence indicates that the ethyl ester is

hydrolyzed more slowly than the methyl ester. There is still less evidence of hydrolysis of n-butyl, isoamyl and secoctyl stearate. These liquid esters remain in the intercellular phase as essentially inert materials. Crystal clefts or cellular responses to the presence of crystals are rarely found. There is a negligible injury to the tissues, and the infiltration with inflammatory cells is so slight that it is hardly worthy of mention.

Results of a corresponding nature follow the use of ethyl oleate, n-butyl oleate and ethyl chaulmoograte. The injury to the tissues and the degree of infiltration with various inflammatory cells are much less conspicuous in the region of injection of ethyl oleate than in that of methyl oleate. Encysted globules of the residual ester are numerous. N-butyl oleate is more inert in the intercellular medium than ethyl oleate. It seems to persist unchanged and excites a minimal response on the part of tissue. Differences similar in magnitude to those in the zones of reaction to methyl oleate and ethyl oleate are also present in the tissues around residual deposits of methyl and ethyl chaulmoograte.

The results which have been mentioned imply that although the methyl esters of certain saturated and unsaturated acids are readily hydrolyzed in the intercellular environment of the subcutaneous tissues, the esters of these acids with higher related primary and secondary alcohols are hydrolyzed very slowly, the rate of hydrolysis being a function of the length of the chain of the esterified alcohol or acid.

It is to be kept in mind that the foregoing statement is only a crude working hypothesis. Many factors certainly play a role. In this regard the experience with a heterocyclic alcohol, tetrahydrofurfurol, esterified with palmitic acid is of interest. This ester is hydrolyzed to a degree comparable with that of the methyl ester of palmitic acid. Crystalline deposits are abundant at the site of injection of the ester. Tissue reactions to crystals are conspicuous. The inflammatory reaction is severe, and few globules of residual liquid ester remain.

Hydrolysis of Synthetic and Natural Glyceryl Esters.—With the knowledge of the behavior of relatively simple esters in the intercellular phase, one may proceed to a consideration of the hydrolysis of glyceryl esters. Some of these are of known structure. The structure of the glycerides which occur in the natural oils is not thoroughly known, although recent work has thrown some light on the configurational relationships of the fatty acids with respect to the glyceryl radicle.[3]

Tristearin and tripalmitin, being solids below 39.5 C., are injected as solids and remain as such in the tissues. The solid glycerides excite fibrosis and a pronounced giant cell reaction. They are highly insoluble, and if they are hydrolyzed at all, it must be a slow process. It is to be

3. Hilditch, T. P., and Jones, E. C.: J. Chem. Soc., London, 1932, p. 805.

recognized that these experiments are not comparable with the others and should be repeated with the glycerides in suitable solvents.

Triolein stimulates such a mild reaction of tissue and so much of the liquid ester remains at the site of injection that with the criteria for evaluating hydrolysis in mind the conclusion is reached that this glyceride is hydrolyzed very slowly, if at all.

Triacetin and tricaproin slowly disappear from the intercellular medium. Triacetin disappears more rapidly than tricaproin and much more rapidly than triolein. The reaction of the tissues is mild around the few globules of encysted residual material. The evidence is rather in favor of slow hydrolysis, but it is to be emphasized that the experiments were of long duration, and the relative solubilities of the different compounds cannot be entirely neglected in the interpretation of the data.

The synthetic glyceryl esters of unsaturated acids isolated from the products of hydrolysis of cod liver oil stimulate reactions which resemble those produced by cod liver oil. The reactions are much less severe than those which follow injections of methyl esters of unsaturated acids of cod liver oil. A considerable amount of encysted liquid material remains in the intercellular region. Furthermore, there are deposits of insoluble amorphous materials such as are found in the regions of injection of cod liver oil. These deposits influence the nature of the inflammatory reaction, and thereby the interpretation of the findings becomes more difficult. It is evident, however, that if hydrolysis occurs, it is slow.

Olive oil persists apparently as an unchanged liquid in the intercellular phase for at least three weeks. It disappears very slowly, and many encysted globules remain at the site of injection. No crystal clefts or cellular reactions to insoluble crystalline solids are found. The glycerides in olive oil have a high percentage of palmitic and stearic acyl groups. The absence of crystalline deposits, the large amount of residual material and the very mild reaction of the tissues indicate that the mixed glycerides of olive oil in general are not hydrolyzed at an appreciable rate.

Linseed oil excites a somewhat more severe reaction than olive oil. The oil has a high content of oleic, linoleic and linolenic acids and a small content of palmitic and stearic acids esterified with glycerol. Most of the oil persists in the intercellular phase as small encapsulated globules of liquid. No crystal clefts are found in the zone of reaction. A small amount of the oil undergoes a transformation into an amorphous insoluble material which stimulates a giant cell reaction. This material is similar to that which is formed in the presence of certain other highly unsaturated compounds, such as those in cod liver oil. The nature of this substance which apparently may form in the presence of intact ester linkages will be considered elsewhere. All evidence indicates that

the glycerides of linseed oil are not hydrolyzed during their residence in the intercellular medium.

The composition of cod liver oil is such that an evaluation of hydrolysis of the glyceryl esters is difficult to make. The oil is composed principally of highly unsaturated glycerides which contain few saturated acyl groups. Other esters than glyceryl esters occur in small amounts. Among these selachyl esters, batyl esters and phosphatides are perhaps the most common. No information is available with respect to the intercellular hydrolysis of these esters. Despite the complexity of the situation, a second order interpretation of the data may be made. The experimental results show that cod liver oil (crude or acid free) stimulates a tissue reaction which is more severe and more complicated than that which occurs at sites of injection of the other oils. The intensity of the reaction increases over a period of at least two weeks. Some liquid material in the form of encysted liquid globules remains in the intercellular phase. These data are most easily interpreted by assuming that the glycerides are hydrolyzed. However, much of the injected material remains in the zone of injection either as an encapsulated liquid or as an amorphous semisolid, relatively insoluble substance. This material resembles the substances of similar physical properties which are found in the regions of reaction to linseed oil and the synthetic glyceryl esters of unsaturated acids of cod liver oil. This material in each instance influences greatly the character of the tissue reaction. It seems to be formed only in the presence of certain highly unsaturated compounds and so far as can be determined, hydrolysis of highly unsaturated esters is not a necessary step in the formation of these insoluble semisolid materials. Therefore, the absence of crystal clefts in the zone of reaction, the moderate amount of residual liquid and semisolid amorphous material and the essential similarity of the histologic changes to those which occur in response to synthetic glycerides of cod liver oil aid in forming the conclusion that the glycerides in cod liver oil, although they undergo interesting physical transformations, are not hydrolyzed at an appreciable rate.

The conclusion in general with respect to the hydrolysis of glycerides in the intercellular medium of the subcutaneous tissues is that if hydrolysis occurs it is a very slow process and is not readily detectable by the methods employed. It is possible, in view of enzyme specificities, that experiments with glycerides of various stereoisomeric configurations may restrict the generality of this conclusion.

Histologic Study of the Tissue Reactions.—The character of the reactions which follow the introduction of an ester into the intercellular phase of the subcutaneous tissues is a function, first, of the composition of the ester; second, of the rate and degree of hydrolysis of the ester;

third, of the composition of the products of hydrolysis, and fourth, of the composition of materials derived by local transformations of primary products of hydrolysis or from transformations of the ester in the presence of an intact ester linkage.

The inflammatory reaction which accompanies hydrolysis of the methyl esters of the homologous series of normal chain saturated acids (C_{10} to C_{18}, excluding C_{15}) reaches a maximum earlier with the esters of short chain acids than with the esters of higher homologous acids. This observation is based on data obtained from inspection of the lesions produced by each ester over a period of two weeks. At the end of two weeks the gross and microscopic studies show that the most intense reaction is at the site of injection of the methyl ester of myristic acid. The lower members of the series do not stimulate such a severe reaction at any time during their residence in the tissues. The higher members of the series are capable of exciting reactions of the same order of magnitude as myristic ester, but as a rule the tissue responses are less active.

There is no difference in magnitude between the response to the methyl ester of an even-numbered carbon chain acid and that to the methyl ester of a comparable odd-numbered chain acid. Furthermore, the rate of hydrolysis of methyl esters of saturated acids with long normal chains seems to be about the same irrespective of whether the acids are natural or unnatural homologues.

The magnitude of the tissue reaction also is influenced by the degree and type of unsaturation of the methyl esters. The response of the tissues and the amount of injury to the tissues in regions where methyl esters of liquid unsaturated acids with 18 carbon atoms (oleic, ricinoleic, linoleic and linolenic acids) are introduced increase with the number of unconjugated ethylenic linkages in the carbon chain of the acid.

The potential effect of alcohols liberated as products of hydrolysis deserves consideration. The possible local toxic action of methyl alcohol cannot be dismissed without some comment. It is to be remembered that a small amount (approximately 0.05 cc.) of this highly soluble alcohol is formed locally over a period of several days during the hydrolysis of each methyl ester. No direct information with respect to the action of the alcohol can be given. Some indirect information is obtained by a comparison of the reactions to tetrahydrofurfuryl palmitate and methyl palmitate. These esters are hydrolyzed at almost the same rate. There is no conspicuous difference in the histologic character of the tissue reactions to the products of hydrolysis of the two esters. Previous studies in which mixtures of glycerol with methyl esters, acids and potassium salts of acids were employed indicate that glycerol has no other effect than to increase slightly the severity of the tissue reactions to the esters, acids or salts.[1] Therefore, the conclusion is reached that

alcohols which may be formed during hydrolysis of esters employed in these experiments do not influence the tissue reaction to a significant degree.

The persistence of unhydrolyzed ester in the intercellular phase may influence the intensity of the tissue reaction in several ways. However, the experimental results show that if the ester is liquid, relatively insoluble in the intercellular medium and free from highly reactive functional groups, it resides, if unhydrolyzed, as an inert substance in the tissue environment. If the liquid ester is highly unsaturated, there is an increased magnitude of the tissue response irrespective of splitting of ester linkages. This is illustrated by the relatively severe reactions to cod liver oil and synthetic glycerides prepared from unsaturated acids of cod liver oil. To a lesser extent this is also true of linseed oil. The increase in the magnitude of the reaction is in part to be attributed to the accumulation of amorphous semisolid, relatively insoluble materials in the intercellular phase. These materials, as well as others which will be discussed elsewhere, arise through transformations of certain unsaturated compounds during their residence in the tissue. It is sufficient at this time to point out the fact that these substances have an important influence not only on the magnitude of the reactions but also on the pattern of cellular responses.

The results of these experiments substantiate previous observations with respect to cytologic reactions.[1] The stimulus to epithelial proliferation is often pronounced adjacent to the zones of reaction to methyl esters of linoleic and linolenic acids. Epithelization of subcutaneous cysts, with partial enclosure of the residual material and exudate by a well formed layer of stratified squamous epithelium, occasionally occurs. This type of epithelial reaction is similar to that which has been described in the zones of reaction to highly unsaturated methyl esters of acids obtained from cod liver oil.

Eosinophils often accumulate in zones of response to highly unsaturated compounds and are invariably rare in the regions of response to saturated compounds.

Macrophages and giant cells with delicately reticulated foamy cytoplasm characterize the reaction to a liquid ester of a saturated acid if the ester is hydrolyzed to yield a highly insoluble crystalline acid which is solid at the temperature of the tissues. Macrophages and giant cells with relatively homogeneous cytoplasm characterize the reactions to the liquid esters of liquid unsaturated acids. There is a positive correlation between the formation of giant cells and the transformation of liquid unsaturated acids or esters into amorphous solid or semisolid products which accumulate in the intercellular phase. Liquid esters of liquid unsaturated acids, whether hydrolyzed or not, do not stimulate a significant giant cell reaction unless this transformation occurs.

The discussion of the experimental results is not complete without proper emphasis being placed on the fact that a complex situation exists in any region where materials are introduced into the intercellular phase. Any induction drawn from the study of the reaction of any compound to the particular environment or vice versa is liable to be faulty. It must be recognized that the questions with respect to histologic changes or to alterations in chemicals which are introduced under one set of conditions in the tissues may be materially different under another set of conditions. This should be true irrespective of the source of the chemical compounds or of the means by which they gain access to the intercellular phase. For this reason, it should be clearly stated, although it is implicit in the data, that one is often dealing here with at least two general sets of conditions which are logically not comparable. For instance, no data which are obtained with respect to compounds that do not produce a significant tissue reaction can be generalized to predict how the same compounds would be affected in any zone of tissue response other than the one specifically investigated.

SUMMARY

Three methods which may be used for the detection of hydrolysis of liquid esters in the intercellular phase of the subcutaneous tissues are presented. The application of these methods to the study of several saturated and unsaturated esters leads to the following conclusions:

Natural and synthetic glycerides which have acyl groups composed of long normal chains of carbon atoms are not hydrolyzed at an appreciable rate.

Glycerides which have acyl groups composed of short chains of carbon atoms slowly disappear from the intercellular medium. If hydrolytic cleavage of the ester linkages occurs, it is not a rapid process.

The methyl esters of long chain saturated and unsaturated acids are rapidly hydrolyzed. The ethyl esters of representative long chain acids are slowly hydrolyzed. The n-butyl esters are not hydrolyzed to an extent readily detectable by the methods employed. Further variation of the alcohol radicle by the use of isoamyl stearate, secoctyl stearate and tetrahydrofurfuryl palmitate shows that the isoamyl and secoctyl esters persist as such in the intercellular environment while tetrahydrofurfuryl palmitate is hydrolyzed rapidly at a rate comparable with that of methyl palmitate.

The study of a partial homologous series of liquid methyl esters of saturated normal chain acids shows: first, that the methyl esters of short chain acids are hydrolyzed more rapidly than the methyl esters of higher homologous acids; second, that there is no conspicuous difference in rate of hydrolysis between the methyl esters of natural acids and the methyl esters of comparable homologous unnatural acids, and,

third, that the formation of multinucleated giant cells in the zones of tissue response to these liquid esters is a consequence of hydrolysis with the liberation of an acid which has a melting point above the temperature of the tissue environment.

The crystallization of acid products of hydrolysis in the intercellular medium sometimes occurs in such a way that there is a parallel orientation of the crystals with their long axes normal to the boundary of the limiting tissues. This arrangement is reminiscent of patterns of crystallization that are often detectable at surfaces in vitro. The palisades of crystals, although not always clearly distinguishable as such, exert a polarizing effect on giant cells, macrophages and fibroblasts, whose cellular processes extend between the crystals for great distances.

The delicately reticulated foamy cytoplasm of macrophages and giant cells is a distinctive feature of the reactions to the liquid saturated methyl esters which are hydrolyzed to yield crystalline acids in the zone of reaction. This cytoplasmic pattern is not a significant feature of the cellular reaction to methyl esters of liquid unsaturated acids.

INTERCELLULAR TRANSFORMATIONS OF UNSATURATED FATTY ACIDS AND ESTERS

AN EXPERIMENTAL STUDY

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Certain oils and fractions of oils which in the natural state are liquid at the body temperature and soluble in alcohol and xylene are transformed during their residence in the intercellular phase of the subcutaneous tissues into insoluble semisolid amorphous substances.[1] Some of these substances gradually acquire the property of retaining phenolized fuchsin with the same tenacity as acid-fast bacilli.

This study was undertaken in an attempt to establish: first, the type of chemical structure which is necessary in any compound in order that such a transformation may occur; second, the general nature of the mechanism which is responsible for the transformation, and, third, the nature of the semisolid insoluble products which are formed.

MATERIALS AND METHODS

The methods of preparation and purification of most of the compounds and mixtures of compounds which were used in these experiments were given in previous reports.[1]

The additional purified materials fulfil the requirements of standard physical constants. The various compounds and mixtures of compounds used in these experiments were as follows:

Natural Oils

1. Olive oil (crude and acid free)
2. Cod liver oil (crude and acid free)
3. Linseed oil (crude and acid free)

Fractions of Natural Oils

1. Nonsaponifiable
   - (*a*) Olive oil
   - (*b*) Cod liver oil
2. Glycerol
   - (*a*) Olive oil
   - (*b*) Cod liver oil
3. Total acids
   - (*a*) Olive oil
   - (*b*) Cod liver oil
   - (*c*) Linseed oil

From the Department of Pathology, Harvard Medical School.

1. Hass, G. M.: Arch. Path. (*a*) **26**:956, 1938; (*b*) this issue, p. 1183.

4. Saturated acids
   (*a*) Olive oil
   (*b*) Cod liver oil
   (*c*) Linseed oil
5. Unsaturated acids
   (*a*) Olive oil
   (*b*) Cod liver oil
   (*c*) Linseed oil

Methyl Esters of Fractions of Oils

1. Saturated acids
   (*a*) Olive oil (one fraction)
   (*b*) Linseed oil (one fraction)
   (*c*) Cod liver oil (three fractions)
2. Unsaturated acids
   (*a*) Olive oil (one fraction)
   (*b*) Linseed oil (one fraction)
   (*c*) Cod liver oil (thirteen fractions)

Sodium, Potassium and Calcium Salts of Total Acid Fractions

1. Olive oil
2. Cod liver oil

Methyl Esters of Saturated Acids of Known Composition

1. Capric acid
2. Undecylic acid
3. Lauric acid
4. Tridecylic acid
5. Myristic acid
6. Palmitic acid
7. Margaric acid
8. Stearic acid

Methyl Esters of Purified Unsaturated Acids

1. Oleic acid
2. Elaidic acid
3. Erucic acid
4. Ricinoleic acid
5. Chaulmoogric acid
6. Linoleic acid
7. Linolenic acid

Higher Esters of Purified Acids

1. Ethyl, n-butyl, isoamyl and secoctyl stearate
2. Tetrahydrofurfuryl palmitate
3. Ethyl and n-butyl oleate
4. Ethyl chaulmoograte
5. Ethyl cinnamate

Glycerides

1. Triacetin
2. Tricaproin
3. Tripalmitin
4. Tristearin
5. Triolein
6. Synthetic mixed glyceryl esters of unsaturated acids of cod liver oil

Compounds Derived from Linoleic and Linolenic Acids

1. Tetrahydroxystearic acid (sativic acid)
2. Tetrabromostearic acid (liquid form)
3. Hexabromostearic acid

Normal Paraffin Hydrocarbons

1. Octane
2. Decane
3. Dodecane
4. Tetradecane
5. Hexadecane

Terminally Substituted Normal Paraffin Hydrocarbons

1. Phenyl and cyclohexyl pentadecane
2. Phenyl and cyclohexyl heptadecane
3. Phenyl and cyclohexyl nonadecane

Miscellaneous Unsaturated Hydrocarbons

1. Styrene
2. Phenyl cyclohexene
3. l-pinene

Young guinea pigs weighing from 250 to 350 Gm. were used. All materials were injected into the subcutaneous tissue of the abdominal wall. The routine dose of liquid materials was 0.1 cc. A few highly irritating substances were given in doses of from 0.05 to 0.025 cc. Compounds solid at the temperature of the tissues were injected in amounts of from 10 to 20 mg. Ether and Tyrode's solution were used as vehicles or solvents for the solid substances.

As a rule the animals were put to death at the end of two weeks. In selected instances the period was extended to three, four and eight weeks. Blocks of the abdominal wall were excised and fixed in solution of formaldehyde U. S. P. (1:10) for from twenty-four to forty-eight hours. Sections including the zones of reaction and residual material were dehydrated in graded alcohols and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin as an aid to recognition of the insoluble material and by the Ziehl-Neelsen method for purposes of demonstrating the acid-fast property.

COMPOUNDS WHICH YIELDED INSOLUBLE AMORPHOUS MATERIALS DURING THEIR RESIDENCE IN THE INTERCELLULAR MEDIUM

The following compounds and mixtures of compounds were partly transformed during a period of two weeks in the intercellular environment into relatively insoluble amorphous semisolid products. In each instance some part of these materials was acid fast.

1. Cod liver oil (crude and acid free)
2. Linseed oil (crude and acid free)
3. Total acids isolated from products of hydrolysis of
   (*a*) Cod liver oil
   (*b*) Olive oil
   (*c*) Linseed oil
4. Unsaturated acids isolated from products of hydrolysis of
   (*a*) Cod liver oil
   (*b*) Olive oil
   (*c*) Linseed oil

5. Methyl esters of unsaturated acid fractions of
   (*a*) Cod liver oil (thirteen fractions)
   (*b*) Linseed oil (one fraction)
6. Methyl esters of purified acids
   (*a*) Linoleic acid
   (*b*) Linolenic acid
7. Glycerides
   (*a*) Mixed glyceryl esters (synthetic) of cod liver oil unsaturated acids.

DESCRIPTION OF THE AMORPHOUS SEMISOLID MATERIALS

The transformation of liquid soluble compounds into the semisolid amorphous insoluble products is gradual. The physical character of the insoluble substances which are formed varies with the nature of the compounds from which they arise. The final localization of the substances is both intracellular and extracellular. The acid-fast property is not acquired by all insoluble materials derived from any given compound or mixture of compounds.

The progressive formation and the variations in the physical character of these amorphous substances are shown most clearly in the studies of cod liver oil. The transformation of the oil is not rapid during the first week in the intercellular phase. During the second and third weeks the insoluble materials appear in relatively large quantities. Some of these materials are still present at the end of eight weeks. They are first detectable as delicate membranes which form at the zone of contact of the oil with the tissues. As a rule the membranes are yellowish, refractile and homogeneous. The homogeneity is often interrupted by the presence of small vacuoles. Occasionally they are fibrillar in appearance, owing in part to the inclusion of condensed strands of fibrin and delicate collagenous fibrils. The membranes as viewed in two dimensions are often broken into shreds, spirilliform filaments and granules. The reasons for the disintegration are not clearly understood. It seems to be brought about principally by the changing physical conditions in the zone of reaction. This is suggested as one reason for the fragmentation of some of the extended, long, continuous membranes, because small globules of oil which are not appreciably disturbed by the mechanical stresses in the tissues often maintain an intact boundary of the insoluble product. In such instances, the boundary zone increases in thickness until the entire globule seems to be transformed into an insoluble substance.

There is a wide variation in the physical character of the amorphous solid products as derived from the several sources. Linseed oil, despite the high average unsaturation of its glycerides, yields much less insoluble material than cod liver oil. The insoluble product is usually in the form of small globules. Membranes are not so conspicuous as they are in the zones of reaction to cod liver oil. The removal of free acids prior

to the injection of each oil has no appreciable effect on the amount or physical nature of the insoluble products which form in the tissues.

The total acid fraction of each oil yields some insoluble material. The total acid fraction of cod liver oil and to a lesser extent the corresponding fraction of linseed oil yield many delicate filaments and small globules of material. A minute amount of a similar insoluble granular substance appears in the zone of reaction to the total acid fraction of olive oil. In each instance the partition of the total acid fraction into saturated and unsaturated acid fractions discloses that the semisolid amorphous products are derived from unsaturated compounds or at least from compounds which are present in the unsaturated fractions.

The operations of esterification and subsequent distillation of the methyl esters of each unsaturated acid fraction result, first, in a loss of the small amount of component or components which serve as a source for the insoluble substances found in the zone of reaction to the unsaturated acids of olive oil, and, second, in no detectable diminution in the capacity of unsaturated acids of cod liver oil or of linseed oil to yield insoluble products during their residence in the intercellular medium.

The physical character of the amorphous substances which form in the zones of injection of the methyl esters of the total unsaturated acid fractions of cod liver oil and linseed oil differs slightly from that of the similar insoluble materials which form in the zones of reaction to the acid-free or crude oils. The membranes and large globules are less conspicuous in the sites of injection of the methyl esters. These deficiencies, at least so far as acids of cod liver oil are concerned, are not corrected by the addition of glycerol to the acids or to the methyl esters of the acids prior to injection of the compounds.

Studies of fractions of the methyl esters of unsaturated acids of cod liver oil show that the amount and physical character of the insoluble material are functions of the boiling point of the esters. The boiling point of these compounds is dependent primarily on the length of the carbon chain of the esterified acid. Inasmuch as the highly unsaturated acids isolated from the products of hydrolysis of cod liver oil have long carbon chains (18-20-22-24 carbon atoms), these acids predominate in the high-boiling fractions. These fractions yield a larger amount of insoluble material than the low-boiling fractions. This material appears in the form of granules and small globules irrespective of the fraction employed. However, shreds and filaments of the insoluble substance arise from compounds which are present only in the high-boiling fractions. These fractions are distinguished chiefly by their high average unsaturation as indicated by the iodine numbers (about 239 to 268 [18]). The amount and physical character of the insoluble amorphous materials,

therefore, are functions not only of the length of the carbon chain but also of the degree of unsaturation of the acid or acyl group of which the chain is primarily composed.

Two purified unsaturated compounds, methyl linoleate and methyl linolenate, are partly transformed into amorphous semisolid insoluble substances during their residence in the intercellular phase. No compound of the same class with a single ethylenic linkage undergoes this transformation. No aromatic compound among those employed in the experiments appears to be amenable to the transformation. This is true even in the instance of styrene, a highly reactive unsaturated hydrocarbon with four conjugated double bonds. Saturation of the ethylenic linkages of methyl linoleate with hydrogen atoms, bromine atoms or hydroxyl groups deprives the compound of its capacity to yield amorphous semisolid products of the type with which we are concerned.

The conclusion is reached that an unconjugated system of at least two ethylenic linkages in an acid or an acyl group having a long normal carbon chain is necessary in any compound which undergoes the described transformation in the intercellular region of the subcutaneous tissues. The presence of more than two unconjugated linkages in the chain favors the transformation. This is illustrated, first, by the larger amount of the substance in the zones of reaction to methyl linolenate than at the sites of injection of methyl linoleate, and, second, by the formation of shreds of insoluble materials in the zones of response to various compounds containing unsaturated acyl groups with three or more unconjugated ethylenic linkages.

All compounds or mixtures of compounds in this series which undergo the gradual change in the intercellular phase have, to a greater or lesser extent, the degree and type of unsaturation mentioned. It is the only chemical characteristic which they have in common other than that the acids or acyl groups have long normal carbon chains. It is assumed here, as is generally accepted, that some linoleic acid is present in the total and unsaturated fractions of olive oil acids.

The products of the transformation are not exclusively localized in the intercellular phase. When they are present in the form of membranes and shreds, they act as a powerful stimulus to the formation of multinucleated giant cells. These cells often attain a large size and not only encapsulate the semisolid products but also incorporate the products in their cytoplasm. The smaller globules and granules, although provoking some formation of giant cells, are more frequently found in the cytoplasm of macrophages. In the intracellular phase as well as in the intercellular medium the insoluble semisolid materials persist for a long time and may be found at the site of injection of various compounds after an interval of two months. So far as can be determined the capacity of the macrophages and giant cells to degrade the substances

is greatly limited. There can be little doubt that the macrophages are operative in the transport of some of the granular materials from the site of formation to regional lymphatics and lymph nodes.

The acid-fast property is not acquired by all of the semisolid insoluble products. Neither do the various products retain the phenolized fuchsin with equal intensity. For these reasons only a general statement is permissible at this time. Some part of all the enumerated compounds or mixtures of compounds which undergo the described transformation gradually acquires the acid-fast property. This property, although variable in degree, is often of the same order as that possessed by the class of acid-fast micro-organisms.

CONSIDERATION OF SOME MECHANISMS WHICH MAY BE OPERATIVE IN THE FORMATION OF THE AMORPHOUS SEMISOLID INSOLUBLE PRODUCTS

The partition of unsaturated compounds amenable to the described transformation from those which under similar circumstances are not so transformed directs attention to some oxidative mechanism as being responsible for the observed alterations in the compounds. It is to be recalled that the most pronounced changes in the nature of these compounds during their residence in the intercellular phase seem to occur in the presence of a particular type and degree of unsaturation of acids or acyl groups with long normal carbon chains. At least two unconjugated double linkages in the chain seem to be necessary. The few compounds with single ethylenic linkages or conjugated systems of double linkages in the series of chemicals employed up to this time do not yield the insoluble semisolid amorphous products.

The chemical behavior of compounds of the type with which one is concerned here under certain conditions in vitro leads to a brief consideration of four general types of oxidation products: first, hydroxy compounds, which are formed by the addition of hydroxyl groups at the double linkages; second, degradation products formed through oxidative splitting of the molecule at the double linkages; third, peroxides, which, according to theory, are formed by the addition of molecular oxygen at the double bonds, and, fourth, polymers of the highly reactive peroxides.

There is indirect evidence that if hydroxy compounds are formed by oxidation of the unsaturated compounds, they do not persist in the subcutaneous tissues in appreciable amounts as long chain polyhydroxy acids or esters. If completely hydroxylated compounds are formed—for instance, dioxystearic, tetraoxystearic and hexaoxystearic acids—such compounds either as acids or as methyl esters should appear as crystalline deposits in the tissues and excite a giant cell reaction. If previous experience in the detection of crystalline deposits may be relied on, the absence of crystal clefts and tissue responses to crystalline deposits in

the zones of reaction to methyl oleate, linoleate and linolenate militates against the possibility that hydroxylation of the double bonds in the absence of other alterations in the unsaturated compounds is important in the mechanism with which one is concerned.[1b] The introduction into the intercellular phase of partially hydroxylated linoleic acid (ricinoleic acid) as a methyl ester should yield a crystalline compound and stimulate a corresponding tissue reaction if there is hydroxylation of the remaining double bond unaccompanied by degradation of the carbon chain. No crystal clefts or tissue reactions to crystalline solids are found in the sites of injection of methyl ricinoleate. That this reaction to crystals in the presence of completely hydroxylated linoleic acid occurs is demonstrable in the zones of reaction to tetrahydroxystearic acid (sativic acid) administered in methyl oleate. Therefore it may be argued that the formation and persistence of hydroxylated acids or of their methyl esters are not of primary importance in the mechanism with which one is concerned, first, because of the absence of crystals and tissue reactions to crystalline deposits in zones of reaction to the unsaturated compounds; second, because the employment of partially or completely hydroxylated linoleic acid as methyl esters or acids yields a negative result so far as the formation of an amorphous insoluble material is concerned, and, third, that hydroxylation of the double bonds of long chain unsaturated acids is not as yet an acceptable step in biologic oxidative degradation.

The experimental results give no proof that oxidative splitting of the carbon chain at the double linkages of the highly unsaturated compounds occurs in vivo. Data which have been reported elsewhere show clearly that the increase in the magnitude of the tissue reaction to fractions of the methyl esters of unsaturated acids derived from cod liver oil is positively correlated with an increase in the average unsaturation of the fraction employed.[1a] This observation is substantiated by a study of the tissue reactions to methyl esters of purified unsaturated acids of similar physical character and of the same length of carbon chain.[1b] Methyl oleate with one ethylenic linkage in the chain excites a mild reaction. Methyl linoleate with two unconjugated ethylenic linkages in the chain causes much more severe necrosis of tissue and inflammatory response. Methyl linolenate with three unconjugated ethylenic linkages in the chain has a destructive action which is more pronounced than that of methyl linoleate. These data suggest that there is greater susceptibility of the more highly unsaturated compounds to oxidation in vivo and that the tissue necrosis is secondary to the local formation of appreciable quantities of the degradation products. If this is true, products arising through oxidative degradation may be implicated in the formation of the insoluble amorphous materials.

The third and fourth general types of oxidation products which may be considered are the peroxides and the polymers of peroxides. The exposure of unsaturated compounds of the type with which one is concerned here to molecular oxygen in vitro and at temperatures within the physiologic range results in partial transformation of the compounds into amorphous, relatively insoluble products. Some of these products have physical characteristics similar to those of the insoluble substances which form in the intercellular environment of the subcutaneous tissues. The accepted opinion with respect to this transformation in vitro is that molecular oxygen adds at the double linkages and that the resulting peroxides polymerize through oxygen bridges to form the insoluble amorphous products. Linoleic acid, linolenic acid and some highly unsaturated acids of cod liver oil are examples of simple compounds which yield these products under suitable conditions in vitro. Such products may form in the presence of intact ester linkages, the double linkages of the acyl groups being the necessary reactive structural units. The formation of these amorphous products is accelerated in vitro by various catalysts, among which several iron salts are efficient.[2] The onset of the auto-oxidation may be delayed in vitro by the presence of antioxidants. However, once the absorption of oxygen has begun, the rate of oxidation proceeds as a rule as if no antioxidant were present. Antioxidants which are capable of retarding this reaction occur widely in nature.[3] The most effective compounds as shown by Mattill are orthohydroxyphenols and parahydroxyphenols, such as hydroquinone and pyrogallol.[3] It is to be noted here that epinephrine belongs to this general class of compounds.

The experimental results show that there is a consistent correlation between a class of compounds which are transformed into amorphous semisolid materials in vitro and unsaturated compounds which undergo a similar transformation in the intercellular phase of the subcutaneous tissues. The prevailing chemical theory with respect to the products which are formed in vitro is that they are composed of polymers of peroxides of the unsaturated compounds. This may permit an expression of opinion that one is dealing here in the tissues with compounds of a similar character. The complex nature and variety of polymers which form under conditions in vitro may be advanced as affording a tentative explanation for the variation in the physical character and acid-fast property of the amorphous semisolid materials which form in the intercellular phase of the subcutaneous tissues. The initial appearance of the insoluble substances at the margins of oil droplets is in accord with the general hypothesis, for it is in this position that contact with oxygen

2. Coffey, S.: J. Chem. Soc. **119**:1152 and 1408, 1921.
3. Mattill, H. A.: J. Biol. Chem. **90**:141, 1931.

or with oxygen donators is most likely. Furthermore, the insoluble acid-fast materials which form in the intercellular phase have a high stability. They persist for long periods in the intercellular medium and are resistant to degradation in the cytoplasm of macrophages and giant cells. The apparent stability of these amorphous products allies them still more closely to the insoluble compounds which are formed in vitro.

The analogies which have been drawn are subject to criticism. This is especially to be emphasized because of the complexity of the intercellular medium and the availability of reactive materials in the intercellular phase. For this reason the possible interaction between mobile or fixed tissue elements and the highly reactive unsaturated hydrocarbons is not to be disregarded. The proof of the validity of the hypothesis depends on isolation and identification of the amorphous semisolid materials which form in the intercellular medium. If previous experience with such complex compounds may be depended on, the identification may be difficult even though the isolation is achieved.

COMMENT

The origin of the products with which one is concerned here and the acid-fast property of some of the materials indicate that they may be related to substances which are responsible for the acid-fast property of certain micro-organisms. In the first place, Anderson [4] has shown that the tubercle bacillus contains a small quantity of linoleic and linolenic acids in ester combinations. It is possible that these compounds may be amenable to the transformations described here. In the second place, tubercle bacilli retain their acid-fast property in the presence of ordinary fat solvents. This is likewise true of the materials encountered in these experiments. In the third place, the only acid-fast material isolated from the tubercle bacillus by Anderson [4] is a saturated nonsaponifiable amorphous wax. The determinations of the molecular weight and structure of this material have offered difficulties, but he has concluded that the compound, which is composed of carbon, hydrogen and oxygen, has a high molecular weight, carboxyl groups, hydroxyl groups and a probable ether linkage. These data offer some support to the speculation that the acid-fast property of the tubercle bacillus may be due in part to the presence of compounds similar to those which arise through transformation of highly unsaturated compounds as described in these experiments.

It may be permissible to mention some general problems which arise in view of the experimental results. It is to be emphasized that under the conditions of these experiments continuous membranes are frequently formed through an apparent transformation of certain liquid

4. Anderson, R. J.: Physiol. Rev. **12**:166, 1932.

compounds. Most of the simple liquid compounds which are amenable to this alteration are of such nature that they may be readily transported in one combination or another in the various bodily tissues. Unsaturated compounds of the type with which one is concerned here, especially linoleic acid, linolenic acid and still more highly unsaturated acids occur free, in ester combinations and perhaps as loose molecular compounds in many cells and tissues of the mammalian body. The metabolism of such materials in normal and pathologic states offers problems which at present are of great interest. Little is known of mechanisms which may be operative in maintaining the integrity of these highly unsaturated compounds. Perhaps, as in the experiments which have been described, the integrity of some of these compounds in the tissue environment is not fully maintained, so that under certain normal and pathologic conditions they undergo transformations in various sites similar to those described here as occurring in the intercellular phase—always influenced by the conditions under which the transformation occurs and the structure of the material to which the unsaturated compound is bound.

SUMMARY

Several liquid unsaturated fatty acids and esters of fatty acids during their residence in the intercellular medium of the subcutaneous tissues undergo a partial transformation into amorphous, semisolid, relatively insoluble materials. The quantity, rate of formation and physical character of the insoluble materials are functions of the structure of the compounds from which they arise. These materials first appear at the interphase of the injected compounds and the regional tissues. They increase in amount with time. They form extended membranes, globules and granules. The final localization of the materials is both intracellular and intercellular. They are highly resistant to degradation through influence of the tissue environment and may persist for at least eight weeks in the intercellular medium. They often acquire the acid-fast property to a degree which is comparable with that to be observed in the class of acid-fast micro-organisms.

So far as these experiments are concerned, at least two unconjugated ethylenic linkages in a long carbon chain of an acid or an acyl group are required before the transformation is possible. If an acid or an acyl group contains more than two unconjugated ethylenic linkages, the structure is favorable for a more rapid and more complete transformation.

The compounds which undergo the described transformation during a period of residence in the intercellular medium are comparable with those which in vitro in the presence of oxygen and under certain other conditions may be transformed into amorphous semisolid, relatively insoluble materials. Such materials, according to theory, are composed of complex polymers of peroxides of the unsaturated compounds.

Reasons are given for believing that the insoluble substances which are formed in the intercellular medium of the subcutaneous tissues arise through the operation of a similar mechanism. A few of the many limitations of this thesis are discussed, with special emphasis on the possibility of entrance of local tissue elements into combination with the highly reactive carbon compounds.

Attention is directed to the possible relationship between acid-fast materials which are formed under the conditions of these experiments and acid-fast materials in certain micro-organisms.

Emphasis is placed on the fact that some unsaturated compounds which are amenable to this transformation occur normally in the tissues of the mammalian body either as acids or acyl groups in diverse ester combinations.

EFFECTS OF ANTERIOR PITUITARY IMPLANTS AND EXTRACTS ON EPIPHYSES AND JOINTS OF IMMATURE FEMALE GUINEA PIGS

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AND
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ST. LOUIS

In former studies we analyzed the effect of acid extracts of anterior lobes of pituitary glands of cattle on the growth of cartilage, bone and bone marrow in immature guinea pigs. We established the following types of action: 1. In the majority of the animals hyperplasia and hypertrophy of the cartilage cells of the epiphysial line occurred, which were soon followed by calcification and replacement of the cartilage by bone. 2. In other animals, hyperplasia predominated, while the process of calcification was retarded.[1]

These disturbances of endochondral ossification were not a local phenomenon, limited to the epiphysial line. We observed similar changes within the joint, where also hypertrophic and degenerative alterations in the cartilage cells took place. Thus arthropathic lesions[2] could be produced similar to those reported in human acromegaly.

Furthermore, it was found in experiments extending over a longer period of time that marked hyperplastic and hypertrophic growth at the chondro-osseous junction of the ribs sometimes led to a rosary-like swelling.[3]

These effects of the extract could be demonstrated in thyroidectomized as well as in nonthyroidectomized animals.[4] This led to the conclusion that the extract acts on cartilage and bone without the intermediation of the thyroid gland.

There still remain the problem as to the specificity of the observed alterations and the problem as to the mechanism underlying them. We

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1. Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **32**:1423, 1935.
2. Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **34**:333, 1936.
3. Silberberg, M., and Silberberg, R.: Proc. Soc. Exper. Biol. & Med. **36**: 622, 1937.
4. Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **33**:554, 1936.

approached the latter problem by a study of the early changes which take place in bone and cartilage under the influence of anterior pituitary substances.

MATERIAL AND METHODS

Altogether the bones of 136 female guinea pigs, littered in the spring, fall and winter, and weighing on the average 180 Gm., were studied. Of these, 14 had received varying doses of acid extracts of anterior lobes of pituitary glands of cattle ranging from 1 to 5 cc., over periods of from one to five days. As a rule, 1 cc. of the extract was injected daily. The animals were killed one or two days after the last injection. Pieces of the anterior lobes of pituitary glands had been implanted in 122 animals. These animals had served in experiments of a different kind carried out by Dr. Leo Loeb, who made it possible for us to study this material in our investigations.

Five animals had only one implant of one fourth of a gland; they were killed on the second day. The remaining 117 animals can be divided into five groups: I. Twelve animals received two implants of one-fourth lobe which were made on two consecutive days; these animals were killed on the fourth day. 2. Twenty-three animals received one-fourth lobe on each of two consecutive days. Examination took place on the fifth day. 3. Ten animals received one-fourth lobe on each of three consecutive days. Examination took place on the fourth day. 4. Eight animals received one-fourth lobe on each of three consecutive days. Examination took place on the fifth day. 5. Sixty-four animals received one-fourth lobe on each of four consecutive days. Examination took place on the fifth day.

In 67 of 117 instances the kinds of cattle from which the anterior lobes of the pituitary glands were obtained had been noted. These lobes included 19 heifer and 19 steer glands, 16 cow glands and 13 bull glands, while 50 lobes were simply designated as from "pituitary glands of cattle." In 27 cases fresh lobes had been used for implantation, while in 95 cases the lobes had been subjected to various chemical treatments prior to implantation; in the latter instances the lobes had been kept for different periods of time in solutions of urea, glycerin, acetone, alcohol, sucrose, solution of formaldehyde U. S. P. and ammonium sulfate or in combinations of these substances.

In every case, at autopsy the knee joint together with the whole tibia was removed for study. The bones were fixed in solution of formaldehyde U. S. P. (1:10) overnight. They were subsequently incompletely decalcified in a mixture of 0.3 cc. of nitric acid and 100 cc. of Müller's solution[5] according to the method of Pommer.[5a] The decalcifying solution was renewed after about four to five days, when it had become dark and opaque. After seven to ten days the material was ready for cutting, provided about 50 cc. of the decalcifying medium had been allowed to act at room temperature; after a frontal section had been made through the middle of the surface of the knee joint by means of a razor blade, the bone was kept for another one or two days in Müller's solution; from there it was transferred into a 5 per cent alum solution in order to prevent the swelling of the tissues which is liable to occur during the process of washing out in running water after decalcification. The specimens were kept in running water for forty-eight hours and subsequently embedded in pyroxylin. Sections were mounted and

5. This is an aqueous solution of 2.5 per cent potassium dichromate and 1 per cent sulfate.

5a. Pommer, G.: Ztschr. f. wissensch. Mikr. **2**:151, 1885.

the pyroxylin was removed according to the method of Maximow [5b] and Rubaschkin.[5c] The sections were stained with hematoxylin and eosin.

OBSERVATIONS

Normal Control Animals.—In completely healthy guinea pigs weighing about 180 Gm. the epiphysial line is invariably patent and of medium width. The zones of (1) the resting, (2) the columnar and (3) the hypertrophic calcifying cartilage are distinct. The individual cell groups consist of euhyaline cartilage cells and are separated by a homogeneous chondromucoid ground substance, which in the part directed toward the epiphysis contains no calcium (fig. 1 [6]).

In accordance with the growth tendency of an animal of this age and owing to a slight extent to the grade of decalcification obtained through the bichromates, this intercartilaginous matrix takes on a more or less bluish stain with hematoxylin, which as a rule is denser toward the distal layers of the cartilage. In the latter area a precipitation of calcium salts becomes noticeable; these salts are deposited first in the form of globules and rods in the vicinity of the hypertrophic cartilage cells, and the details of the cell structure remain as yet preserved. Still more distally, a calcium incrustation of the cells themselves takes place; the cell walls are broken down by the advancing bone marrow, and at last the cells are replaced by bone.

The chondrophyte—i. e., the lateral protuberance of the epiphysial line—shows a somewhat different structure and mode of reaction. It does not participate in the calcification to the same extent as the epiphysial line proper. More centrally the chondrophyte is also composed of typical euhyaline cartilage cells, while toward the periphery there are layers of a more undifferentiated type of cartilage, and finally, adjoining the perichondrium, there is a tissue resembling ordinary connective tissue. The abundant ground substance of the chondrophyte reveals acidophil properties, which increase toward the periphery, thus demonstrating a collagenous nature of these tissue elements which is different from the chondromucoid of the epiphysial cartilage.

In the cartilage covering the joint three layers can be distinguished: (1) the outer, sliding zone, consisting of flattened small cells; (2) the transitional zone, with chiefly rounded cells, for the greater part horizontally arranged, and (3) the pressure zone, containing the hypertrophic cartilage cells which lead into the zone of ossification. The cartilage cells are separated by a richly fibrillar acidophilic nonmucoid intercellular stroma.

5b. Maximow, A.: Ztschr. f. wissensch. Mikr. **26**:177, 1909.

5c. Rubaschkin, W.: Anat. Anz. **31**:30, 1907.

6. Dr. H. A. McCordock made the photomicrographs.

Experimental Animals.—After a single implantation of one fourth of the anterior lobe of a pituitary gland or after one injection of 1 cc. of an extract of anterior lobe, the structure within the epiphysial line remains almost normal. Only in 2 cases of implantation was perhaps a trace of swelling of the chondromucoid matrix seen.

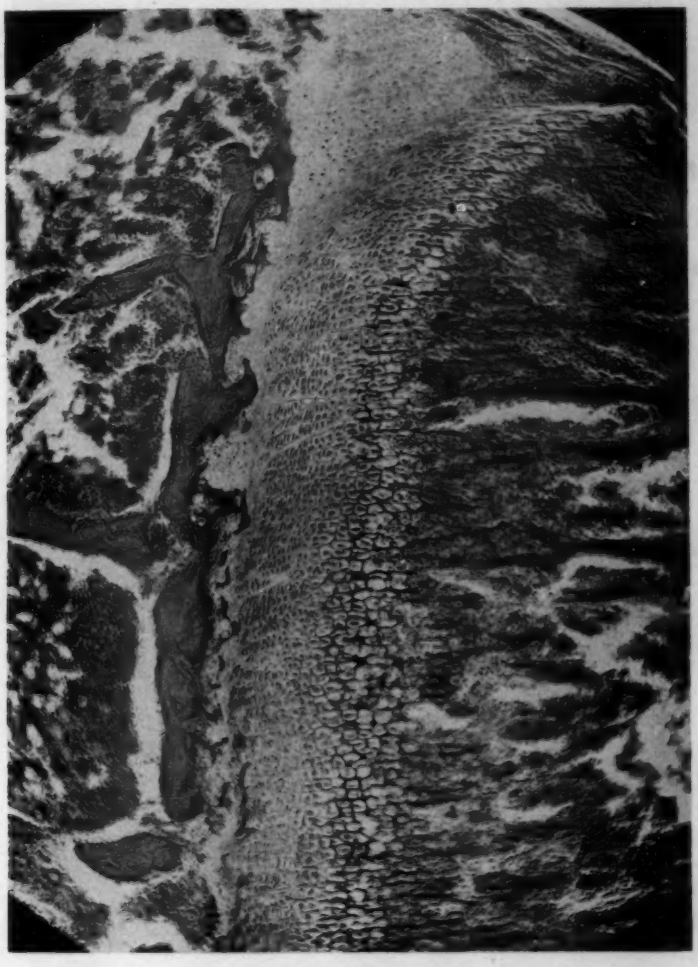

Fig. 1.—Zone of endochondral ossification in a normal female guinea pig weighing 180 Gm.; medium magnification. The different layers of cartilage cells can be readily recognized. There is a moderate amount of chondromucoid matrix and of deposited calcium. Slender ossified trabeculae are seen. The triangular chondrophyte is resting.

After two implantations of one-fourth lobe or two injections of an extract of anterior lobe made on two successive days, the first

definite changes may be observed. These become, however, more pronounced if the implanted or injected substances are allowed to act on the animal for from one to three days longer before the tissues are removed for examination. The earliest lesions are detected within the matrix of the epiphysial cartilage. The chondromucoid ground substance appears increased, is swollen and takes on a more basophilic

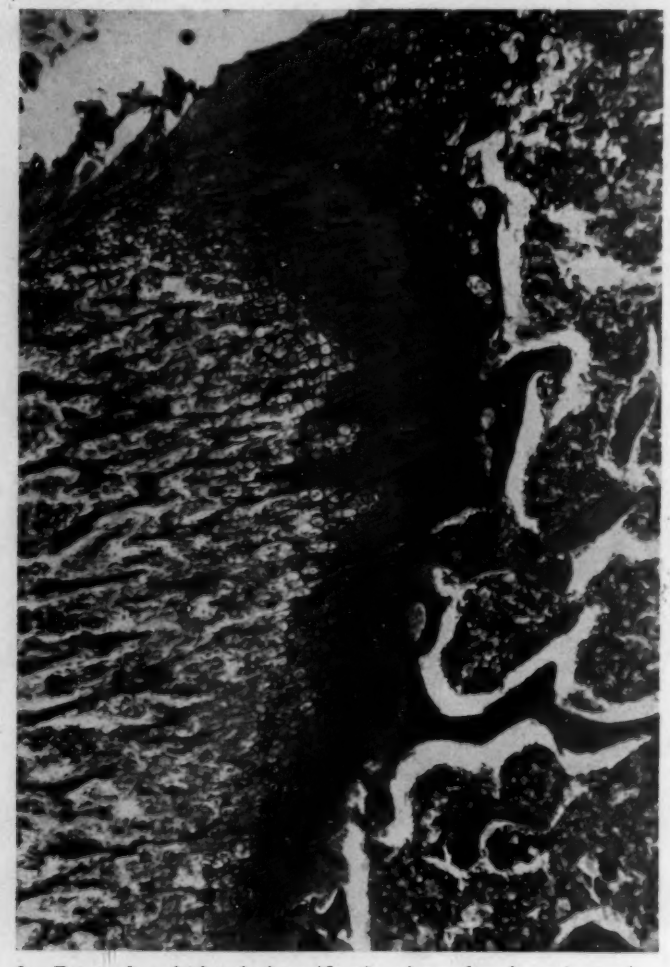

Fig. 2.—Zone of endochondral ossification in a female guinea pig weighing 180 Gm. which had received on four successive days four implants of anterior lobe from the pituitary gland of a cow, one-fourth lobe being used each time; examination took place on the fifth day; medium magnification. Extensive degeneration and cell débris are seen throughout the epiphysial line, leading to destruction of whole rows of cartilage cells. The triangular chondrophyte is hypertrophic. This represents a medium effect.

stain. In such areas a dense network of fibrils within the ground substance becomes visible. These fibrils are separated from one another by dustlike granular or linear precipitations of basophilic amorphous masses. As the changes advance, the intercartilaginous stroma becomes more vacuolated and at last dissolved. After four or five days, there

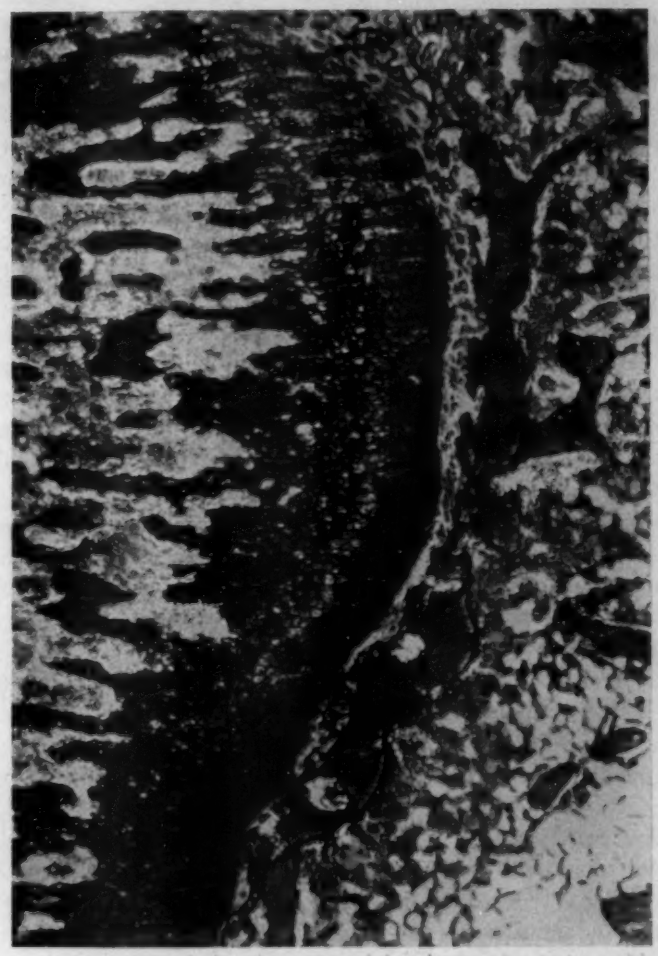

Fig. 3.—Zone of endochondral ossification in a female guinea pig weighing 180 Gm. which had received on four successive days four implants of anterior lobe from the pituitary glands of cattle, one-fourth lobe being used for each implantation; examination took place on the fifth day; medium magnification. There are seen marked calcification of the entire epiphysial zone and dense deposits of calcium in the thickened trabeculae. Details of the cellular structure of the narrowed epiphysial line cannot be recognized. Large "incubator capsules" are seen in particular on the top, the innermost, part of the chondrophyte. Fibrosis of the bone marrow is noted. This is an example of a strong effect.

may appear within the epiphysial disk wide gaps and cavities filled with a mucoid liquid which stains slightly metachromatically. Two types of cellular changes can be associated with these lesions. The first one is retrogressive in nature (fig. 2): The large nuclei of the resting and columnar cartilage cells become smaller or pyknotic; the finer structure of the nuclei and nucleoli disappears, and the cytoplasm becomes vacuolated and in the end is destroyed. In some places remnants of these cells, staining diffusely reddish with eosin, are visible, whereas in others shadow-like contours of degenerated cells may still be observed. Gradually the disintegrated or shrunken cells perish, while absorption and liquefaction of this material still progress. The second type of change is of a proliferative nature and may be found without any symptoms of retrogressive alterations: The resting cartilage cells become more numerous, chiefly by way of amitotic cell division, and the interstitial stroma appears, therefore, relatively diminished. The small dense cell nuclei, each surrounded by a wide area of lighter staining cytoplasm, are oval and constricted. These changes take place distally as well as proximally. In cases in which these changes are pronounced, two or more distinctly basophilic cells are surrounded by a dark blue-staining capsule. The cells have not only increased in number; they have in addition undergone hypertrophy. The columnar cartilage cells have likewise increased in number and in size. However, in the majority of cases the retrogressive changes are more pronounced than the proliferative ones. They may be observed in different parts of the epiphysial zone, and not only single cartilage cells but entire cell groups may be destroyed, so that in the end whole rows of cartilage cells are lacking.

In some instances only the retrogressive changes are found. In others, however, there is at the same time a beginning growth of the different cells. In still others, growth of the cartilage is stimulated without evidence of retrogressive changes. Owing to the varying degrees of hyperplasia and hypertrophy, the normal proportions of the various cartilaginous zones may be altered inasmuch as columnar and hypertrophic cartilage may appear to be increased while the layer of the resting cartilage cells is relatively narrow.

After implantation of one-fourth lobe, or after injection of 1 cc. of an acid extract of anterior lobe on each of three or four successive days, the vacuoles and cavities in the chondromucoid ground substance and the cell débris gradually disappear, while considerable amounts of calcium are deposited in the degenerated as well as in the preserved chondromucoid. These deposits appear as basophilic amorphous masses, which assume the shape of globules and finally form disconnected lines or lattice-like figures, taking on a dark bluish stain with hematoxylin. This deposition of calcium takes place first in the periphery of the

cells. Meanwhile, the resting and columnar cartilage cells continue to proliferate and hypertrophy, and subsequently the hypertrophied cartilage undergoes calcification and ossification. In the majority of cases the calcification is accomplished with greater rapidity than the new formation of cartilage, and as a result of this condition the epiphysial line becomes narrower than it is normally. After this stage has been reached, it is but one step to the dense calcification of the epiphysial line, on one hand, and the extraordinary hypertrophy and hyperplasia of the cartilage cells, on the other, which we reported in our first experiments and which can now be reproduced by implanting pieces of anterior lobes of pituitary glands of cattle on six or eight successive days, one-fourth lobe being used daily (fig. 3).

In some instances, however, only degenerative processes can be detected, no subsequent calcification following. They may or may not be accompanied by increased growth of the cartilage cells, and in these cases the epiphysial line may retain a medium width. Lastly, in 7 of 122 cases, i.e., in 5.7 per cent, no changes could as yet be discovered in these early periods.

The course which the changes take within the chondrophyte differs in some respects from that within the epiphysial line proper. Generally, degenerative processes within the intercellular substances do not occur in this area, and the cell proliferation becomes manifest only after implantations or injections have been made on four or more successive days. Under these circumstances the less differentiated peripheral cells, which resemble connective tissue cells, become converted into precartilaginous cells with greater rapidity, and the interstitial matrix is diminished in amount. Simultaneously a characteristic hypertrophy takes place in the zone of the mature cartilage cells: The nuclei of the latter become larger, the cells assume a spheroid shape and are closely pressed together, and four or more such cells are enclosed in a capsule, which is strongly basophilic in its staining reaction. There results thus a formation of cartilage cells which are markedly hypertrophic; they assume a dark blue stain with hematoxylin, and thus the appearance of typical "incubator capsules" is produced, similar to those which have been described in human material under various proliferative conditions of the cartilage (Erdheim [7]; Moritz [8]). As a consequence of this hyperplastic and hypertrophic growth, large basophil cartilaginous buds grow into the epiphysis.

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7. Erdheim, J.: Die Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie, in Aschoff, L.; Elias, H.; Eppinger, H.; Sternberg, C., and Wenckebach, K. F.: Pathologie und Klinik in Einzeldarstellungen, Berlin, Julius Springer, 1931, vol. 3.

8. Moritz, A. R.: Virchows Arch. f. path. Anat. **267**:746, 1928.

In cases in which the calcification of the epiphysial line is less pronounced, the cellular changes within the chondrophyte are also less marked.

As regards joints, as early as after four daily implantations of pieces of anterior lobe or after four injections of the extract of anterior lobe

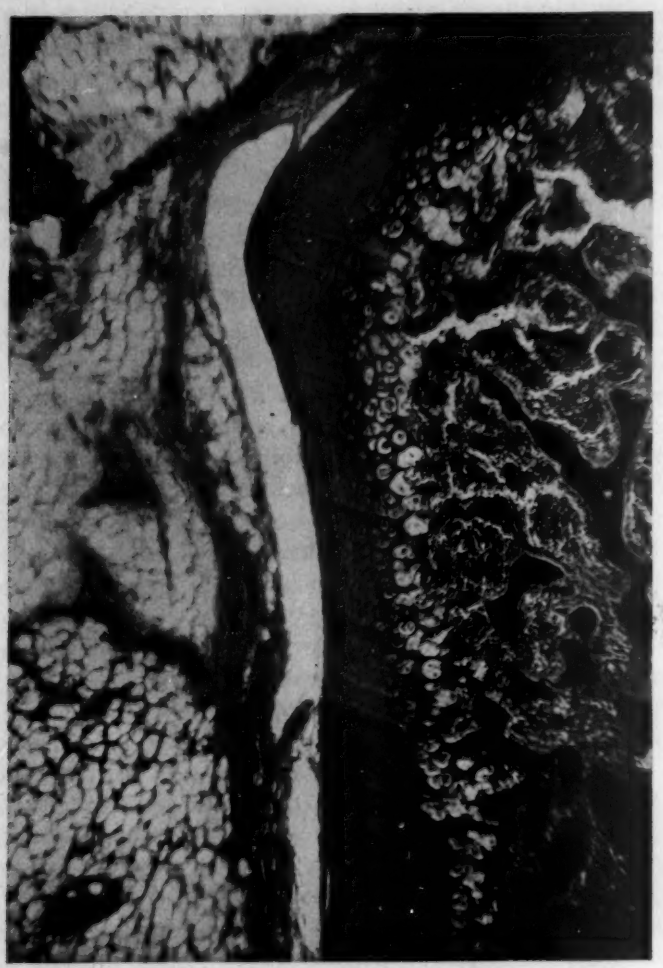

Fig. 4.—Joint surface of the tibia of a normal female guinea pig weighing 180 Gm.; medium magnification. The various cartilage cells are in a resting condition.

a noticeable amitotic multiplication of the cartilage cells occurs, in particular within the transitional zone (figs. 4 and 5); as a result of these proliferative processes, the cells, which individually still possess their normal structure, take on a perpendicular arrangement, the nuclei are

lightly stained, and they include distinct nucleoli; furthermore, four or more of these cells are surrounded by one larger capsule. The flatter cells of the sliding zone likewise proliferate, while the cartilage of the pressure zone appears rather hypertrophic. These changes are still more pronounced after six or eight daily implantations or injections,

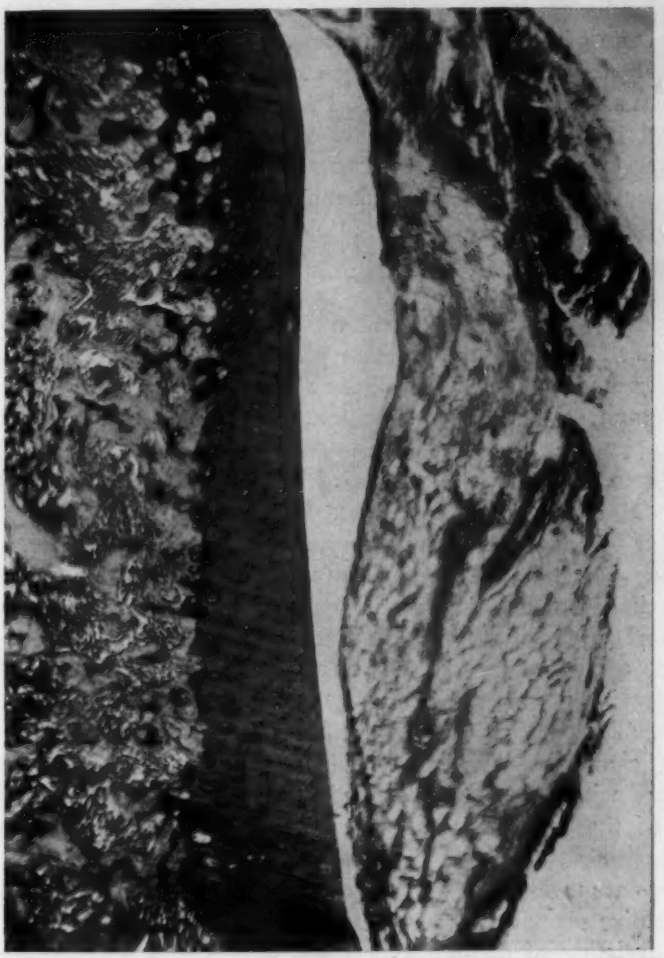

Fig. 5.—Joint surface of the tibia of a female guinea pig weighing 180 Gm. which had received on eight successive days eight implants of anterior lobe from the pituitary glands of cattle, one-fourth lobe being used for each implantation; examination took place on the ninth day; medium magnification. The sliding cartilage is hyperplastic. Cartilage cells of the transitional zone are distinctly hyperplastic and somewhat hypertrophic and show a perpendicular arrangement. Increased ossification is shown.

when the cytoplasm of the transitional cartilage cells may become hypertrophic and more lightly staining; concomitantly with the increase in the number of the cartilage cells and their hypertrophy, the intercellular substance diminishes, although degenerative changes are lacking in the intercellular stroma of the joint. Ossification of the hypertrophic cartilage is increased. Here and there, proliferative processes in the cartilage cells of the joint are found without any signs of preceding degeneration of the epiphysial cartilage.

The bone marrow is invariably congested. In the most proximal areas it has a fibrous character. In cases in which the changes are more advanced, strands of hypertrophic calcified cartilage are seen extending far into the fibrous marrow (fig. 3). There is a faint indication of the outlines of the trabeculae, but ossification has not taken place. In the bone marrow there may also be some mitotic proliferation of undifferentiated blood cells; likewise in the perichondral cells mitoses may occur.

We find thus as an early result of the administration of preparations of anterior lobes from the pituitary glands of cattle a variety of effects in cartilage cells and in intercellular stroma, consisting in degenerative changes and deposits of calcium salts as well as in proliferation and hypertrophy of cells. Certain degrees in the intensity of these changes can be seen to be as a rule associated in different cases, and it is therefore possible to distinguish the following gradations.

Grade 0 is assigned in cases in which no effect is noticeable.

Grade 1 corresponds to a weak effect: Swelling and degeneration of the chondromucoid intercartilaginous matrix go hand in hand with a trace of precipitation of calcium and other basophilic material in the the zone of the resting and columnar cartilage, together with atrophy or destruction of single cells or of whole rows of cartilage cells.

Grade 2 represents a medium effect: Retrogressive changes affecting the chondromucoid ground substance and the cartilage cells are associated with a pronounced basophilic state and a generalized incrustation of the intercartilaginous ground substance with calcium salts, which extends through all the cell layers of the epiphysial line, which thus becomes more narrow the more the calcification progresses. These retrogressive changes are associated with or followed by proliferative ones, consisting of definite hyperplasia and beginning hypertrophy of the resting and columnar cartilage cells. Proliferative and hypertrophic changes become likewise noticeable in the cells of the chondrophyte, in which retrogressive changes in the intercellular matrix are lacking.

Grade 3 represents a strong effect: Deposition of large amounts of calcium salts is associated with narrowing of the epiphysial zone. The infiltration with calcium salts not only affects the cartilage cells peripherally but extends even into their cytoplasm. The finer cell

structures as well as the normal configuration of the epiphysial line therefore disappear. Parallel with these changes, marked hyperplasia and hypertrophy of the cells of the chondrophyte take place together with an accelerated conversion of the more undifferentiated elements into precartilaginous and cartilaginous cells and the formation of typical basophilic cartilaginous incubator capsules. At this stage, the earliest growth and hypertrophy, particularly of the cells of the transitional zone of the joint cartilage, may be observed. In cases in which the changes are far advanced, fibrosis of the bone marrow, extending far into the zone of the hypertrophic calcifying cartilage, may also be seen.

It was of interest to determine whether correlations exist between the different grades given to the tissue changes, on one hand, and the amount of anterior pituitary substance which was implanted and the length of time during which the latter was allowed to act, on the other. In this connection we also considered the treatment to which the anterior lobes of the pituitary glands had been subjected previous to implantation,

TABLE 1.—*Effects of Implantation of Pieces of Fresh Anterior Lobe of Pituitary*

Implants	Day of Examination	Guinea Pigs	Guinea Pigs Showing			
			No Effect	Weak Effect	Medium Effect	Strong Effect
1	2d	4	2	2 ? (perhaps trace)	0	0
3	4th	4	0	2	2	0
2	5th	11	1	0	6	4
3	5th	3	0	0	2	1
4	5th	5	0	0	0	5

as well as the sex and age of the animals from which the glands had been obtained. Finally, we tried to determine whether correlations exist between the changes which we observed in the epiphysial line and the changes which according to Loeb [9] and his collaborators occur in the ovaries as a result of the implantation. The latter investigations have so far been only partially published.[10]

Dr. Loeb suggested that we make use of his findings and records.

The effects of implantation of fresh glands are shown in table 1. From the data presented in this table four conclusions follow: 1. One implant of one fourth of a lobe acting for one day is practically ineffective. 2. Implantation of one-fourth lobe on each of three consecutive days with examination on the fourth day results in a stronger effect than one single implantation and in a weaker effect than three

9. Loeb, L.; Anderson, H. C.; Saxton, J.; Hayward, S. J., and Kippen, A. A.: Science **82**:331, 1935.

10. Loeb, L.; Saxton, J., and Hayward, S. J.: Endocrinology **20**:511, 1936. Hayward, S. J., and Loeb, L.: Proc. Soc. Exper. Biol. & Med. **36**:250, 1937. Loeb and others.[9]

implantations with examination on the fifth day. 3. Three implantations of one-fourth lobe on three successive days, respectively, with examination on the fifth day are somewhat more effective than two implantations of one-fourth lobe acting for the same length of time but

TABLE 2.—*Effects of Implantation of Portions of Anterior Lobes Which Had Been Subjected to Various Kinds of Treatment Before Implantation*

Implants	Day of Examination	Guinea Pigs	Guinea Pigs Showing			
			No Effect	Weak Effect	Medium Effect	Strong Effect
1	2d	1	0	1 ? (perhaps trace)	0	0
2	4th	13	0	4	6	3
3	4th	6	0	1	5	0
2	5th	11	0	3	6	2
3	5th	5	0	0	2	3
4	5th	59	6	5	23	25

TABLE 3.—*Effects of Implantation of Pieces of Anterior Lobes of Pituitary Glands Obtained from Different Types of Cattle*

Type of Cattle	Guinea Pigs	Guinea Pigs Showing			
		No Effect	Weak Effect	Medium Effect	Strong Effect
1. Two Implants, Examination on Fourth Day					
Heifer	2	0	1	1	0
Steer	2	0	0	2	0
Bull	4	0	1	2	1
Cow	5	0	2	1	2
2. Two Implants, Examination on Fifth Day					
Heifer	8	0	1	6	1
Steer	8	1	1	3	3
Bull	2	0	0	2	0
Cow	4	0	1	1	2
3. Three Implants, Examination on Fourth Day					
Heifer	3	0	2	1	0
Steer	3	0	1	2	0
Bull	3	0	0	3	0
Cow	1	0	0	1	0
4. Three Implants, Examination on Fifth Day					
Heifer	1	0	0	1	0
Steer	1	0	0	0	1
Bull	1	0	0	1	0
Cattle (nonclassified)	5	0	0	2	3
5. Four Implants, Examination on Fifth Day					
Heifer	5	0	0	4	1
Steer	5	0	0	1	4
Bull	3	0	0	0	3
Cow	6	0	1	2	3
Cattle (nonclassified)	45	6	4	16	19

somewhat less effective than four daily implantations of one-fourth lobe followed by examination on the fifth day. 4. Implantation of one-fourth lobe on each of four successive days with examination on the fifth day is the most effective procedure.

We may then conclude that two factors determine the degree of the reaction following implantation of fresh anterior lobe from the pituitary glands of cattle: (1) the amount of substance implanted and (2) the time during which it is able to act on the tissues of the host.

In table 2 results are shown which were obtained after implantation of portions of pituitary anterior lobes which had been subjected to various kinds of treatment previous to implantation.

As far as the effects of variations in the amount of implanted gland and in the length of time during which it acts are concerned, the treated and the untreated anterior lobes on the whole behave similarly.

TABLE 4.—*Effects of Implantation of Pieces of Anterior Lobes of Pituitary Glands of Cattle in Guinea Pigs in Which Ovaries Showed Mature Follicles Without or With Very Slight Luteinization*

Implants	Day of Examination	Guinea Pigs	Guinea Pigs Showing			
			No Effect	Weak Effect	Medium Effect	Strong Effect
2	4th	8	0	1	4	3
3	5th	4	0	0	1	3
4	5th	35	3	3	11	18

TABLE 5.—*Effect of Implantation of Pieces of Anterior Lobes of Pituitary Glands of Cattle in Guinea Pigs in Which Mature Follicles Were Not Observed but in Which Luteinization Was Promient*

Implants	Day of Examination	Guinea Pigs	Guinea Pigs Showing			
			No Effect	Weak Effect	Medium Effect	Strong Effect
1	2d	5	2	3 (trace)	0	0
2	4th	5	0	3	2	0
	5th	22	1	3	12	6
3	4th	10	0	3	7	0
	5th	4	0	0	3	1
4	5th	29	3	2	12	12

In table 3 anterior lobes of pituitary glands obtained from different types of cattle are compared as to effectiveness. The results do not indicate that the kind of cattle from which the anterior lobes of pituitary glands have been obtained influences their effect on the epiphysial line and joint to any noticeable degree.

Table 4 represents cases in which mature follicles were present while the processes of luteinization were lacking or very slight in the ovaries.

Table 5 represents the degrees of reaction in the epiphysial zone in those cases in which mature follicles were not observed but in which as a rule the processes of luteinization were prominent.

We may conclude that no definite parallelism exists between the kinds of effects which these anterior pituitary substances exert on ovaries and their ability to produce changes in the various constituents of the epiphysis and of the joint.

COMMENT

Retrogressive changes which take place in the epiphysial layer represent a very early response of this tissue to the hormones of the implanted pieces of anterior lobes of the pituitary glands of cattle or of the injected extracts.

It has been seen that under the influence of a substance or of substances obtained from the anterior lobes of pituitary glands of cattle degenerative changes take place in the cartilage of the joint at a later period than in the epiphysial cartilage and that they are entirely lacking in the chondrophyte. It might seem surprising that these three types of cartilage, which are so similar in their structure and situated near to one another, should react so differently toward the same substances. It is, however, necessary to consider the fact that the interstitial substances of the chondrophyte and joint differ in consistency and also presumably biochemically in certain respects from the chondromucoid of the epiphysial line as indicated by the difference in staining reactions of these tissues, to which we have referred in the first part of this paper. Furthermore, the cells which compose these various tissues differ in their degree of differentiation.

A further problem concerns the relation which exists between the early degenerative changes described by us and the subsequent phenomena of growth. Must it be assumed that the tissue defects initiate the subsequent proliferation and hypertrophy of the cartilage cells, which thus would represent a kind of regenerative growth, or is the cell proliferation a direct response to the growth stimulus given off by the anterior lobe of the pituitary gland? In this connection, it seems important to note that the stimulation to growth in the chondrophyte and joint cartilage occurs independently of degenerative processes in these tissues; degeneration may be lacking altogether or may follow the growth processes in these tissues. Moreover, even in the epiphysial line growth processes may in some instances take place without preceding degenerative changes having been noticeable. We may therefore conclude that in all probability degenerative processes are not a necessary condition for the occurrence of the hyperplastic and hypertrophic changes in the cartillage which we have described and that the latter are due to direct stimulation by substances given off by the anterior lobe of the pituitary gland and are not regenerative in character.

A similar question may be asked concerning the relation between the retrogressive changes and the process of calcification in the epiphysial line. It can be shown that a certain percentage of the treated animals did not exhibit any tendency toward calcification, although the cartilage cells may have increased in size and number. It is possible that in such cases the retrogressive alteration in the epiphysial line had undergone repair and was therefore no longer noticeable at the time when growth processes occurred. Likewise no abnormalities in the calcification of the epiphysial line were noticeable in a certain percentage of cases, and in these, premature calcification and closure of the epiphysial line did not occur. It is therefore hard to prove that a direct relation exists between the early degenerative changes and the subsequent processes of calcification.

As to the changes in the bone marrow, the occurrence of fibrosis in the latter, reaching far into the zone of hypertrophic cartilage in place of the normally lymphoid tissue, may be secondary to the changes which take place in and near the epiphysial cartilage and in the adjoining bone.

It is furthermore of interest to note that the changes which we have observed as the result of implantation of pieces of anterior lobes of pituitary glands of cattle or following injections of extracts of such lobes are entirely different from those seen in the course of other disturbances of the endochondral ossification, such as those which occur in rickets or certain other diseases.

That degenerative processes of so pronounced a nature may take place so soon after the implantation of pieces of anterior lobes of pituitary glands is not surprising in view of the fact that cartilage and bone may respond very readily to various influences. Thus, in cases of deficiency of vitamin C (Wolbach[11]), in cases of some infectious diseases or in cases of underfeeding of animals (Harris[12]) retrogressive changes in the cartilage could likewise be observed as early as after seventy-two hours. Proliferative processes, however, have not been reported under the latter circumstances. As to the nature of the anterior pituitary substance which is responsible for the observed changes, we have not been able so far to identify it with the thyroid-stimulating factor or with any of the substances which induce specific changes in the ovary; the changes occurred as readily in cases in which luteinizing processes predominated in the ovary as in those in which fully mature follicles were observed in the ovary.

Cartilage and bone are tissues possessing a relatively simple structure. It may therefore be foreseen, perhaps, that different kinds of

11. Wolbach, B. T.: Science **86**:569, 1937.

12. Harris, H. A.: Brit. J. Radiol. **4**:561, 1931.

stimuli or irritations may induce changes which show a certain similarity. Further investigations must show whether the changes which we have described following the action of anterior pituitary substances, in particular also the growth processes and the production of incubator capsules, represent a specific effect of the anterior pituitary hormones or whether similar reactions may be produced also by certain other glands with internal secretion.

SUMMARY

Anterior lobes of pituitary glands of cattle as well as extracts of these lobes contain one or more substances which exert two main effects on the epiphysial cartilage of immature female guinea pigs:

1. A swelling of the chondromucoid matrix, followed by or associated with atrophy and degeneration of whole rows of cells in the epiphysial cartilage. In certain cases such changes may disappear after some time, and the cartilage may thus be restored to normal, but in the majority of instances these degenerations are associated with or followed by very extensive calcification.

2. Hyperplasia and hypertrophy of the cells in the different layers of the epiphysial cartilage, the chondrophyte and the cartilaginous covering of the joint.

In the greater number of animals the proliferative changes in the epiphysial line are preceded or accompanied by the aforementioned retrogressive processes. In other instances, however, proliferation may occur without preceding retrogressive alterations; this is the rule in the cartilage of the chondrophyte and the joint.

We may therefore conclude that the growth of cartilage in all possibility is a direct response to the stimulation exerted by a substance which is present in the anterior lobe of the pituitary gland rather than a regenerative process caused by degenerative changes.

While the amount of active substance administered and the time during which it acts on the various kinds of cartilage influence the degree of the reaction, they do not determine the relative predominance of the principal effects which we have described. No correlation could be established between the changes noted in the tissues of the joint and the epiphysial line and the effects exerted on the ovary of the guinea pig. Anterior lobes of pituitary glands which had been modified by chemical treatment in such a way that they differed in their action on the ovaries, some inducing luteinizing processes, other maturation processes, exerted the same effects on the epiphysial and joint cartilage, and conversely, anterior lobes which exerted the same effects on the ovaries could differ in their action on the tissues of the epiphysis and of the joint.

No evidence could be obtained which indicated that the anterior lobes of pituitary glands of different kinds of cattle, such as the steer, heifer, cow and bull, differ in their action on the cartilage.

The histologic characteristics of the various tissues in the epiphysis and in the joint which are observed at different periods following the application of preparations of anterior lobes of pituitary glands are determined by the degrees of intensity with which growth and degenerative processes, calcification and ossification take place.

Case Reports

MULTIPLE ANEURYSMAL FORMATIONS ASSOCIATED WITH RHEUMATIC CARDITIS

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Director of Laboratories, St. Vincent's Hospital

This paper presents the results of a study of an unusual case of diffuse vascular disease resulting in the formation of multiple aneurysms in every large or medium-sized artery of the body. In the literature are to be found examples of multiple aneurysms located in the aorta and in the pulmonary, carotid, innominate, subclavian, brachial, iliac, femoral, renal, splenic, hepatic, mesenteric, gastric radial, ulnar and cerebral vessels. In every reported instance the aneurysms were circumscribed while the wall of the vessel between and beyond them was normal. It is in this respect that the present case is unique, for practically every portion of the arterial system examined appeared diseased both to the unaided eye and microscopically.

REPORT OF A CASE

A 49 year old woman entered Bellevue Hospital, second medical division, Nov. 8, 1934. Her illness had begun insidiously five months before with increasing weakness, progressive dyspnea and gradual loss of vision ending in blindness of the left eye.

The family history was irrelevant. From her past history no evidence of rheumatic fever or of syphilis was elicited.

She appeared chronically ill. The pupils were dilated, irregular and fixed to light and accommodation. The fundus disclosed sclerotic tortuous vessels and pale optic disks. Fine rales were heard at the base of the left lung. The heart appeared to be enlarged. At the apex and base were systolic and diastolic murmurs. The rhythm was regular and the rate normal; the blood pressure was 160 systolic and 0 diastolic. The abdomen was normal except that the liver was palpable 1 inch (2.5 cm.) below the right costal border. There was no edema or clubbing of the fingers. An irregular red-brown lesion covered the legs, the thighs and the backs of both hands. The older lesions were covered by thin scales; healed, they formed pigmented scars.

The most amazing finding was the visible and palpable aneurysms of all peripheral vessels: both external carotid arteries (two aneurysms in each), brachial arteries (two in each), femoral arteries (three in each), right and left posterior tibial arteries (four in each). A bruit could be heard by placing the stethoscope over the patient's left eye.

The patient grew increasingly weak and died eighteen days after admission. Throughout she had a low fever.

The specific gravity of the urine was 1.020; the albumin content, 1 plus. Microscopic examination of the urine gave negative results repeatedly.

From the Laboratories of Pathology and the Second Medical (Cornell) Division, Bellevue Hospital.

The blood showed: nonprotein nitrogen, 36 mg. per hundred cubic centimeters; red cells, 4,000,000 per cubic millimeter; hemoglobin content, 80 per cent; white cells, 9,000, with polymorphonuclear leukocytes 80 per cent, lymphocytes 14 per cent and mononuclear cells 6 per cent. The Wassermann reaction of the blood was repeatedly negative. The spinal fluid showed a trace of sugar; the Lange colloidal gold curve was negative for syphilis; the Wassermann reaction was negative.

Roentgen examination revealed enlargement of the heart and aneurysmal dilatation of the ascending aorta. The electrocardiogram showed a normal tracing.

Necropsy.—The body was that of a 49 year old normally developed but poorly nourished woman, weighing approximately 110 pounds (49.9 Kg.) and measuring 62 inches (157.5 cm.), with mild edema of the ankles and lesions of the skin over the lower extremities, some purpuric, others crusted, but the majority pigmented brown and having the form of a circle about 1 cm. in diameter.

The peritoneal cavity contained 300 cc. of fluid. The liver extended 9 cm. below the costal margin. There was about 100 cc. of fluid in the pleural sacs.

The right lung weighed 390 Gm.; the left, 510 Gm. Both were congested, particularly in the dependent portions. The liver weighed 2,220 Gm. and had a nutmeg appearance. The gallbladder was completely filled by a cauliflower-like growth. The spleen, weighing 450 Gm., was enlarged, firm and tense. The adrenals, pancreas, gastrointestinal tract, kidneys, bladder, uterus and tubes were unchanged. The ovaries were small and sclerotic; the cervix, lacerated. The trachea, thyroid and parathyroids appeared to be normal. No changes were noted in the vertebrae, the sternum or the bones of the calvarium.

The heart was enlarged, owing to hypertrophy and dilatation of all chambers. The tricuspid ring admitted three fingers. Its leaflets were thin and flexible. At the base of the commissure between the septal and anterior cusps were prominent blood vessels. The chordae tendineae of the adjoining leaflets were short and hugged the adjacent thickened ventricular endocardium. The leaflets of the pulmonary valve looked natural. One of the commissures was slightly separated. Over each of the three commissures were small raised hyaline plaques. The pulmonary artery and its branches, including the intrapulmonary vessels, were dilated and exhibited the same intimal changes as were observed in the aorta. The auricular endocardium above the right commissure of the mitral valve was ridged as seen in cases of mitral insufficiency. The ridges were not large enough to be called endocardial pockets, however. The leaflets of the mitral valve were diffusely and moderately thickened, flexible and vascularized. Several small aneurysms bulged above the surface of each. A few chordae tendineae of the posterior leaflet were thickened. The leaflets of the aortic valve were slightly and irregularly thickened. All the commissures were separated to a slight degree, and over each were small raised hyaline plaques. The posterior cusp contained one small aneurysm and the right anterior cusp two. There was slight stenosis of the ostium of the right coronary artery. Both coronary vessels were patent throughout, the wall of the left being slightly thickened.

The ascending aorta was markedly dilated, especially its right wall, which formed one large diffuse aneurysm. Beyond the arch, dilatation was less marked, and the aneurysms were small, measuring 8 mm., but numerous. The walls of the entire vessel and its branches were in addition thin and nonelastic. The entire intima presented a wrinkled, puckered appearance and occasional raised silvery plaques. The yellow of the underlying media had given way to a gray tissue. No normal surface was seen except for an irregular patch in the arch which was still smooth and thin and revealed the normal yellow of the media.

The renal, splenic and iliac arteries and the great vessels of the neck showed the same diffuse dilatation and surface changes. The femoral, popliteal, subclavian and brachial vessels presented a similar appearance.

At the base of the brain two large aneurysms occupied the ophthalmic arteries. The right measured 3 by 2 by 1.5 cm.; the left was half that size. These aneurysms

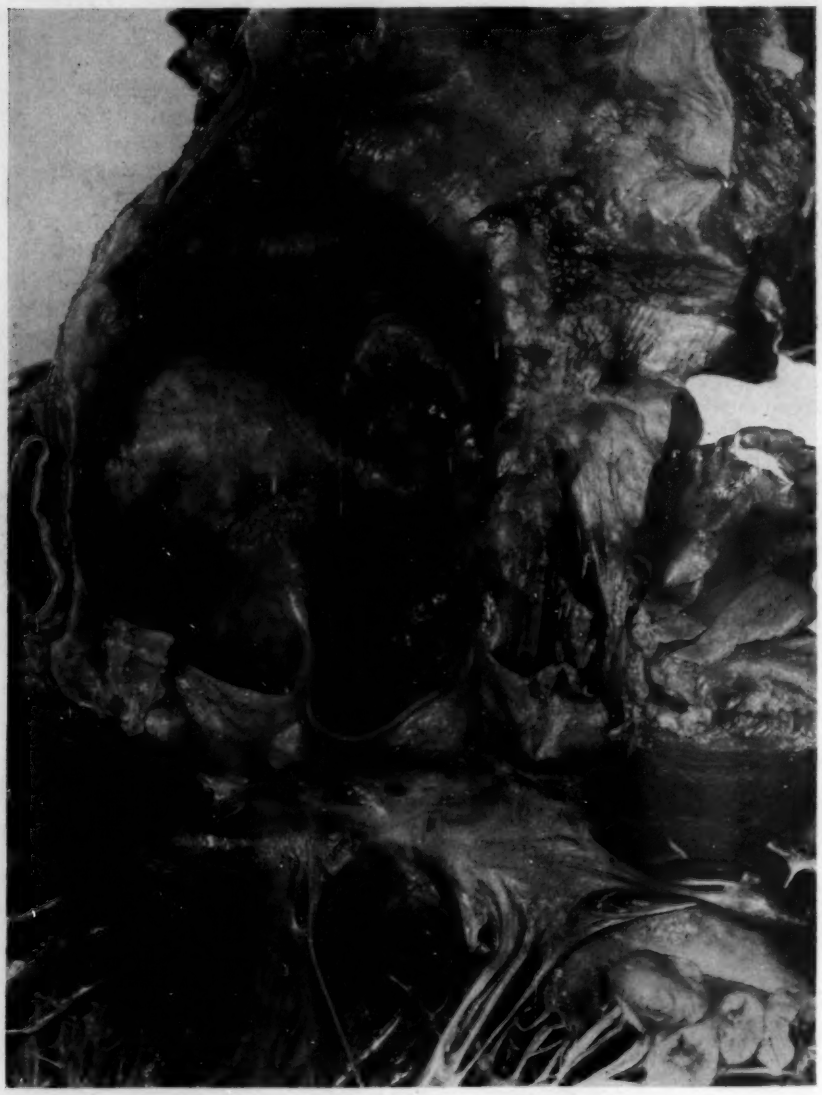

Fig. 1.—Heart and aorta. Note the small aneurysms on the aortic and mitral valves. The ascending aorta is the seat of a large aneurysm. Observe the wrinkling and puckering of the intima. Similar changes were noted throughout the arterial system.

displaced and thinned the optic nerves and eroded the bones about the optic foramina and sphenoid sinus. On section no changes were found in the brain or spinal cord. The pituitary gland was compressed.

Microscopic Examination.—Sections were cut from all valve leaflets and from the myocardium after the method of Gross, Antopol and Sachs.[1] Numerous

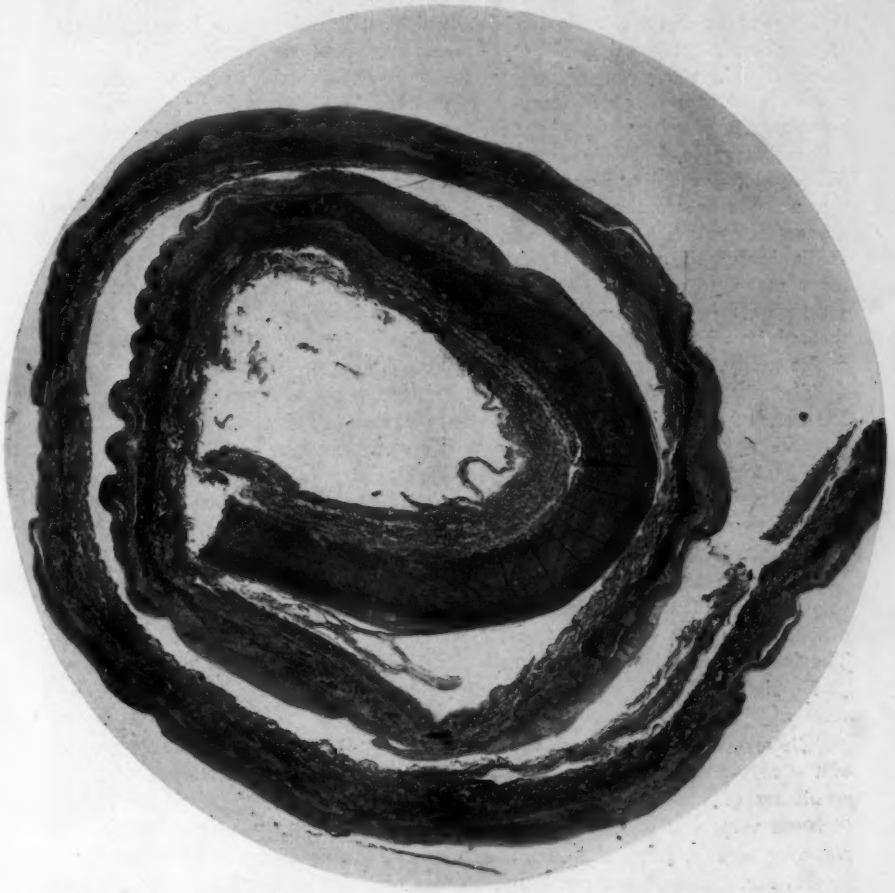

Fig. 2.—Longitudinal section of the entire ascending aorta and arch. The changes are principally in the ascending portion. Note the thickening of the intima and adventitia with the narrow media between. The elastic laminae are frequently interrupted and repaired by vascularized fibrous tissue. Weigert's stain for elastic tissue.

sections were cut from every portion of the aorta, both in the transverse and in the longitudinal direction. Many were made through the pulmonary artery and its branches, the great vessels of the neck, the splenic, renal, mesenteric, hepatic, uterine, ovarian, common iliac, femoral and popliteal arteries, the vessels at the

1. Gross, L.; Antopol, W., and Sachs, B.: Arch. Path. **10**:840, 1930.

base of the brain and finally through the aneurysm of the ophthalmic artery. In addition, all organs were fixed in a solution of formaldehyde, embedded in paraffin, sectioned and examined microscopically. The following stains were used: Hematoxylin and eosin; Van Gieson's stain for connective tissue; Weigert's stain for elastic tissue; Mallory's phosphotungstic acid–hematoxylin stain and finally thionine as a stain for metachromatic changes in the aorta. The Levaditi technic was used for possible demonstration of spirochetes.

The intima of the aorta was diffusely thickened and contained numerous large thick plaques composed of dense fibrous tissue with only traces of fat and no calcium. The media was severely and extensively altered. Large portions of the normal components of this layer were replaced by richly vascularized scars. In some areas the entire width of the media was so replaced. Short narrow fragments of persisting elastic tissue were all that reminded one of the original structure. Where not so completely destroyed, the elastic lamellae were crowded together into a dense narrow structure between the thickened intima and the adventitia. In some areas the latter two coats were in actual contact, the elastica and muscle of the media having disappeared. Inflammatory cells, represented by lymphocytes and large mononuclear cells, varied in numbers. The adventitia was fibrotic. The intima of the vasa vasorum was thickened, narrowing the lumens. Scattered throughout were numerous lymphocytes.

Levaditi stains for spirochetes showed none.

The microscopic changes in the great vessels of the neck and in the iliac, renal, femoral, popliteal, splenic and pulmonary vessels were essentially the same as those in the aorta. In all these vessels they were severe and advanced.

Histologically, the wall of the cerebral aneurysm was composed of a thin dense layer of fibrous tissue free from elastic fibers. The lumen of the sac was filled with a thrombus. No unusual changes other than intimal sclerosis were demonstrated in either the nearby or the more distant cerebral vessels.

The only small artery in which inflammatory changes were found in the media was one located in the serosa of the esophagus. Here were seen thinning of the media, loss of elastic tissue, fibrosis, vascularization and round cell infiltration. The intima was thickened by fibrous tissue, while the adventitia was unchanged.

No similar changes were observed in the hepatic, ovarian, mesenteric or cerebral vessels.

The myocardial fibers were well preserved, for the most part. In some sections of the myocardium one saw perivascular fibrosis. Many typical Aschoff bodies, consisting of small groups of large mononuclear cells, were observed adjacent to blood vessels in some instances and in areas free from vessels in others. The coronary vessels, large and small, were essentially normal. The endocardium in several sections contained patches of fibrosis and small collections of lymphocytes.

The aortic leaflets were slightly and diffusely thickened. The elastic laminae were frayed and in places absent. Thick vessels coursed far out from the base of the cusps. Occasional focal areas of endothelial cell proliferation were noted near the surface, while more deeply located were small nests of loosely distributed lymphocytes. In the aneurysmal pouches the central collagenous core was thin. As the elastic lamina approached the proximal margin of the aneurysm it stained less intensely, disappeared and then reappeared beyond the distal margin near the free border of the cusp.

Both leaflets of the mitral valve were diffusely thickened by increased amounts of connective tissue. The elastic laminae were fragmented and in the aneurysms were completely absent. The chordae tendineae at their junction with the under-

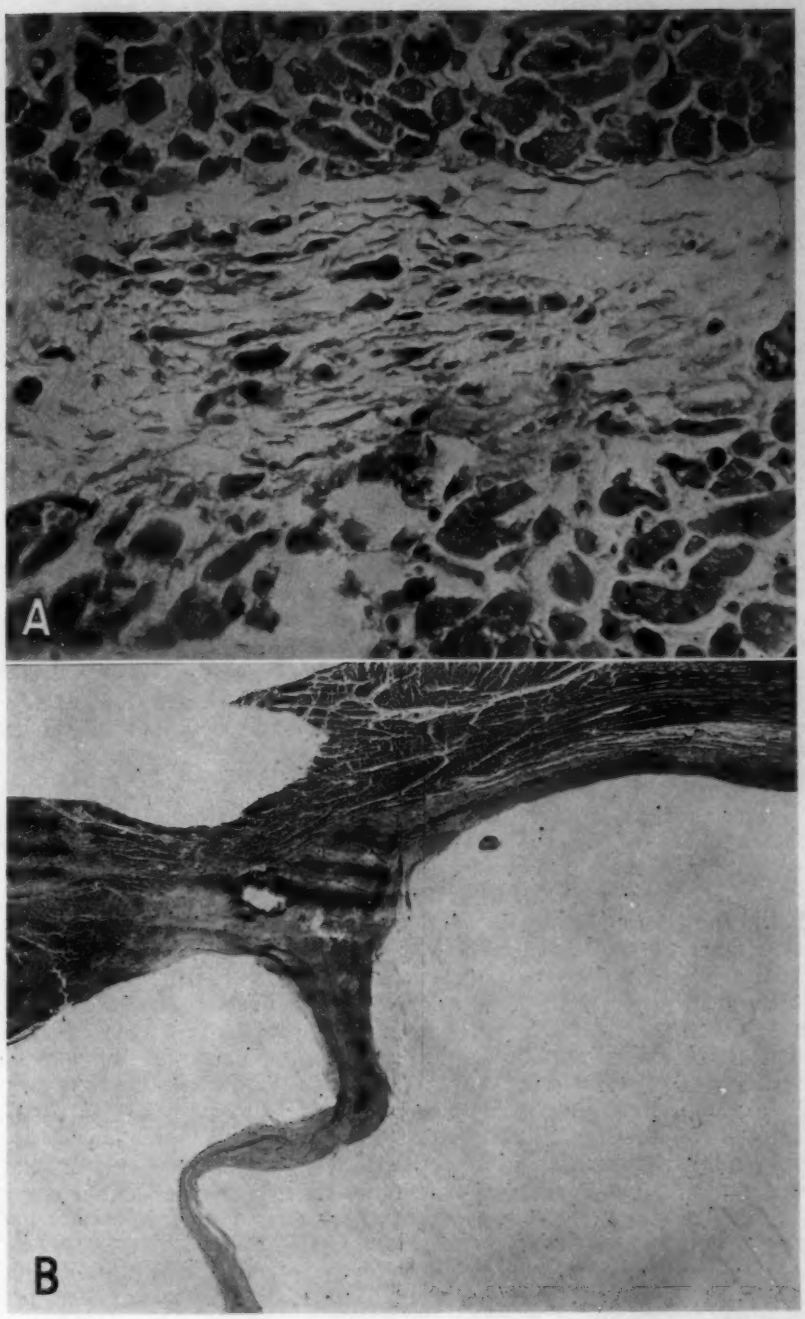

Fig. 3.—*A*, Aschoff body from the left ventricle; hematoxylin and eosin. *B*, section through an aneurysm of an aortic leaflet; hematoxylin and eosin. Note the gradual thinning. The elastic lamina thins, becomes lighter, frays and then disappears.

surface of the valves were not only fibrosed but also the seat of histiocytic proliferation.

The free border of each leaflet of the tricuspid valve was thickened. The septal leaflet in addition contained blood vessels with thick walls. Deep in its substance lymphocytic infiltration was present.

The cusps of the pulmonary valve were normal.

Sections from the lung revealed no pathologic changes affecting the parenchyma or small vessels. The same was true of sections of the pituitary, adrenal and thyroid glands. The kidneys were normal. A mild degree of elastic tissue reduplication was noted in the interlobular vessels. The ovaries were sclerotic; the tubes, normal. The endometrium was hemorrhagic. The uterine cervix contained many small cysts. In the liver chronic passive congestion with central necrosis was noted. The growth in the gallbladder was a benign papilloma. The spleen appeared much congested, and the gastrointestinal tract was unchanged.

Sections were made through the lumbar and upper cervical regions of the cord. With none of the stains employed was it possible to demonstrate changes.

A section taken through a lesion of the skin showed a thin and atrophied epidermis, ulcerated at one point. The corium was infiltrated diffusely with large round cells. The blood vessels were normal.

Anatomic Diagnosis.—The primary conditions were cardiac hypertrophy and dilatation, chronic active inflammation of the mitral and tricuspid valves, inactive inflammation of the aortic valve, multiple aneurysms of the mitral and aortic valves, separation of the aortic commissures, stenosis of the ostium of the right coronary artery, active rheumatic carditis, syphilis of the aorta and of the pulmonary and peripheral vascular systems and multiple aneurysms.

The general observations were: peritoneal, pleural and pericardial effusion, hypostasis of the lungs, chronic passive congestion of the liver, splenomegaly and papilloma of the gallbladder.

COMMENT

The unusual feature in this case was the extensive destruction of the media of practically all extraparenchymal arteries, leading to weakening of their walls and universal formation of aneurysms.

"Aneurysms" were likewise found in two cusps of the aortic valve and in the anterior leaflet of the mitral valve. Whereas in the valvular aneurysms the sole change was thinning of the wall with loss of elastica, elsewhere in the same cusps, as well as in other cusps free from aneurysms, there was a chronic inflammatory reaction, most pronounced on the under surface of the mitral valve at the junction with the chordae tendineae. In addition, a moderate number of Aschoff bodies were found scattered through the myocardium.

The question of cause naturally arises. Clinical efforts to establish the cause were fruitless. The changes visible to the naked eye and the microscopic changes in the aorta were those of syphilis. Typical as they were, one hesitates momentarily to regard them as such, for the usual lesion in syphilis is focal, or if lesions of this disease are widespread, there still remain large portions of the vascular tree which are unaffected. A widespread involvement such as that seen in this case must be rare. Efforts to find a similar example in the literature were unrewarded.

Concerning the aneurysms in the cusps of the aortic and mitral valves, the first temptation is to ascribe their cause to the factor

responsible for the lesion in the aorta, namely, syphilis. However, in the few cases reported in which valvular disease was caused by syphilis,[2] the lesion consisted of diffuse thickening of the leaflets, the presence of vascularized scars and coagulation necrosis with epithelioid, giant and lymphocytic cell reaction. Finally in these cases there was the ability to trace the lesion from the root of the aorta directly into the affected aortic cusp or into the anterior leaf of the mitral valve. In the case reported now, while severe and active lesions were traceable to the roots of the aorta and pulmonary artery, they stopped abruptly at the annulus. Furthermore, the inflammatory changes were more appropriately ascribed to rheumatism because (1) they appeared like it histologically and (2) the Aschoff bodies in the myocardium were typical as were also the endocardial lesions of the chordae tendineae. Although rheumatic changes were present in the valves, one hesitates to say that they led to the formation of the valvular aneurysms, since the usual change produced by rheumatic fever is a thickening rather than a thinning of the valves. Thus one is left in the dilemma of choosing between two improbable causes, unless one wishes to complicate the issue further by raising the question of congenital weakness in the valves.

Some thought was given to rheumatism as the cause of the aortitis and peripheral arteritis. As described by Klotz[3] and confirmed by Pappenheimer and Von Glahn,[4] the aortic lesions in rheumatism are usually microscopic; if they are visible to the naked eye, they are still small. No one has as yet described vascular involvement as severe as this in rheumatic fever.

Medionecrosis aortae in the sense of Gsell[5] and Erdheim[6] is not to be considered in this case, for the disease as described is characterized by destruction of the media, complete absence of cellular reaction and failure of dense fibrous scar formation.

SUMMARY

A case is reported of aneurysms involving the entire arterial system. The histologic lesion in the aorta appeared to be syphilitic. Also present were rheumatic myocarditis and valvulitis.

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ATROPHY OF THE PANCREAS WITH EXTREME FATTY METAMORPHOSIS OF THE LIVER AND STEATORRHEA

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The case now reported is of signal importance not only because of its rarity but more particularly on account of its bearing on certain recent experimental studies. Moreover, this case seems to shed some light on the nature and causes of sprue and on other related but obscure problems in the metabolism of fat.

REPORT OF CASE

Except for typhoid fever at the age of 25 and a hysterectomy which had been done for excessive bleeding twenty-one years before, the past health of the patient, a white woman 68 years old, had always been good. Two children were living and well. The husband died fifteen months before from coronary sclerosis, and the patient had been depressed since his death. In the latter part of October 1936 a cold developed. It got better but was followed by severe diarrhea. From the first there were from four to six stools daily; the stools were yellowish, bulky, semisolid, offensive and floated in water. There were brief periods of improvement followed by relapses, and this course continued for about four months until the woman's admission to the hospital Feb. 7, 1937. Early in this illness there had been some "constricting" abdominal pain, which was neither typical nor well localized. At the times when the stools were more frequent, there were sharp abdominal cramps.

The usual weight before the illness in question was 135 pounds (61.3 Kg.). March 10, she weighed 96½ pounds (43.7 Kg.); March 18, 97½ pounds (44.2 Kg.) and on March 29, 98 pounds (44.5 Kg.). The weight at death, July 14, was 104 pounds (47.2 Kg.) (a considerable edema had developed).

The patient was weak, dejected and depressed. The pulse rate was 90; the blood pressure was 135 systolic and 70 diastolic. The tongue was red and atrophic, with several aphthous ulcers. The heart and lungs were essentially normal. The abdomen was moderately distended, and borborygmi were heard.

Roentgen examination of the gastrointestinal tract April 1 showed no abnormality except segmentation and pooling of the barium sulfate in the small bowel.

By April 6 the patient had begun to present considerable edema of peripheral and dependent parts and this increased somewhat and continued until death

The urine on February 10 had a specific gravity of 1.009, no albumin and no sugar; on July 6 it had a specific gravity of 1.013, albumin (3 plus) and no sugar.

The examinations of the blood gave results as follows:

	February 12	April 1	June 17
Hemoglobin, per cent.....	81	75	67
Red blood cell count.....	5,050,000	4,700,000	3,660,000
Leukocyte count.....	9,400	6,150	7,250
Differential count:			
Polymorphonuclears, per cent	67	73.5	82
Lymphocytes, per cent.....	30	25.5	14
Monocytes, per cent.....	1		2
Eosinophils, per cent.....	1		1
Basophils, per cent.....	1	1	1

Morphologically, the red cells and leukocytes were normal.

The Wassermann reaction of the blood was negative. Analysis of the blood January 21 showed values for the blood components as follows: sugar, 126 mg.; urea, 14 mg.; calcium, 10.25 mg.; phosphorus, 3.45 mg., per hundred cubic centimeters.

The gastric contents January 21 showed no free hydrochloric acid.

The stools were gray to light brown, revealed no blood and contained variable amounts of undigested fat.

The patient was kept on a diet low in fat and roughage. She was given cod liver oil, brewers' yeast and liver extract, and several blood transfusions were made. Opiates and other symptomatic remedies were used in an effort to control the diarrhea.

There was no good response to any of the therapeutic measures, and the patient died on July 14, about eight months after the first appearance of symptoms.

Autopsy.—The body was that of a well developed, poorly nourished white woman, 154 cm. in length and weighing 104 pounds. Rigor was beginning to appear (the autopsy was made 1 hour post mortem); hypostasis was faintly evident over the posterior parts; there was no cyanosis and no jaundice. There was a pitting edema of grade 3 in both lower extremities below the inguinal ligaments and an edema of grade 1 in the left hand.

The peritoneal cavity contained about 2,000 cc. of clear straw-colored fluid. The diaphragm reached to the fourth interspace on the right and to the fifth on the left.

Each pleural cavity contained about 1,500 cc. of clear straw-colored fluid. There were no adhesions in the right pleural cavity; the lower lobe on the left side was densely adherent to the diaphragm. The pericardial sac contained no excess of fluid and no adhesions.

The heart weighed 180 Gm. There was a moderate amount of normal-appearing adipose tissue in the epicardium. The myocardium was brown but otherwise showed no gross lesion. The endocardium was normal. The root of the aorta showed no atherosclerosis. The coronary vessels were normally patent at their orifices, and no points of narrowing could be seen along their trunks.

The right lung weighed 250 Gm.; the left, 300 Gm. No areas of consolidation were present in either lung, but crepitation was markedly reduced in both. On section the surfaces were relatively dry and had a fleshy appearance. No pus could be expressed.

The spleen was densely adherent to the diaphragm by strong fibrous adhesions. It weighed 30 Gm.; its capsule, except for adhesion tags, was smooth. On section the malpighian corpuscles were apparent, and the trabeculae, which were prominent, were close together.

The liver was densely adherent to the diaphragm by strong fibrous adhesions; in removing the liver parts of the diaphragm had to be cut away. The under surface of the liver was adherent to the omentum and to the mesocolon. The liver weighed 1,175 Gm.; its capsule was smooth except where adhesions were present. The liver was yellow-brown. On section the hepatic parenchyma had a definite fine nutmeg appearance; tiny brown dots and lines were surrounded by broader yellow areas. The gallbladder contained about 90 cc. of thin yellow bile, in which a few sandlike concretions were present. The bile ducts appeared normal.

The gastrointestinal tract was carefully examined from the esophagus to the anus. The duodenal mucosa appeared normal, and the papilla of Vater was readily identified. In the ileum several tiny dark submucous hemorrhages, about 3 mm. in diameter, were noted. No gross atrophy of the mucosa of any part was noted, and no ulcers were found. There was no abnormal dilatation of any segment of the bowel.

The pancreas weighed 50 Gm. The pancreas was much smaller than normal, and the normal pancreatic lobulation was entirely gone. The parenchymal lobules seemed to have been replaced by an increased amount of adipose tissue and by bands of dense fibrous tissue. In fact, the pancreas was recognized only by the gross form and position of this fatty, fibrotic mass. On section the pancreas had a grayish yellow hyaline appearance with no lobular structure. Dilated pancreatic ducts were apparent on the cut surfaces. About these ducts tiny, flecklike golden yellow areas were seen against the background of the hyaline fibrous tissue and the lighter yellow fat. In the head of the pancreas, immediately adjacent to the duodenum in the region of the papilla of Vater, was a nodular mass of greater density than the remainder of the gland. This mass measured about 1.5 by 1 cm. in diameter and was adherent to the duodenum. Section through it showed a firm slightly granular grayish white tissue.

The right kidney weighed 60 Gm.; the left, 50 Gm. The capsules stripped with ease, leaving smooth surfaces. Sections showed normal markings. The pelves and ureters were normal. The bladder showed a number of superficial ulcers, from 3 to 5 mm. in diameter.

The uterus was absent. Both ovaries were present as firm small nodule-like structures, measuring about 1 cm. in diameter. In the right ovary a number of small cysts were present.

The aorta showed atherosclerosis of grade 1.

The thyroid was grossly normal. Two parathyroids were isolated and appeared grossly normal. They weighed 25 and 35 mg., respectively.

There was no lymphadenopathy.

The head was not examined.

Microscopic Examination.—The description of the pancreas may be conveniently divided into two parts.

1. Sections taken through the duodenal wall and the ampulla of Vater (fig. 1 *A*) showed that the canal of the ampulla was intact, as were also the duodenal mucosa and the glands of Brunner. There was infiltration of the tissues subjacent to the ampulla by an adenocarcinoma of a scirrhous type. Although the infiltration involved the muscular layers of the duodenum, it had caused no ulceration and had not invaded the ampulla. It appeared to have had its origin

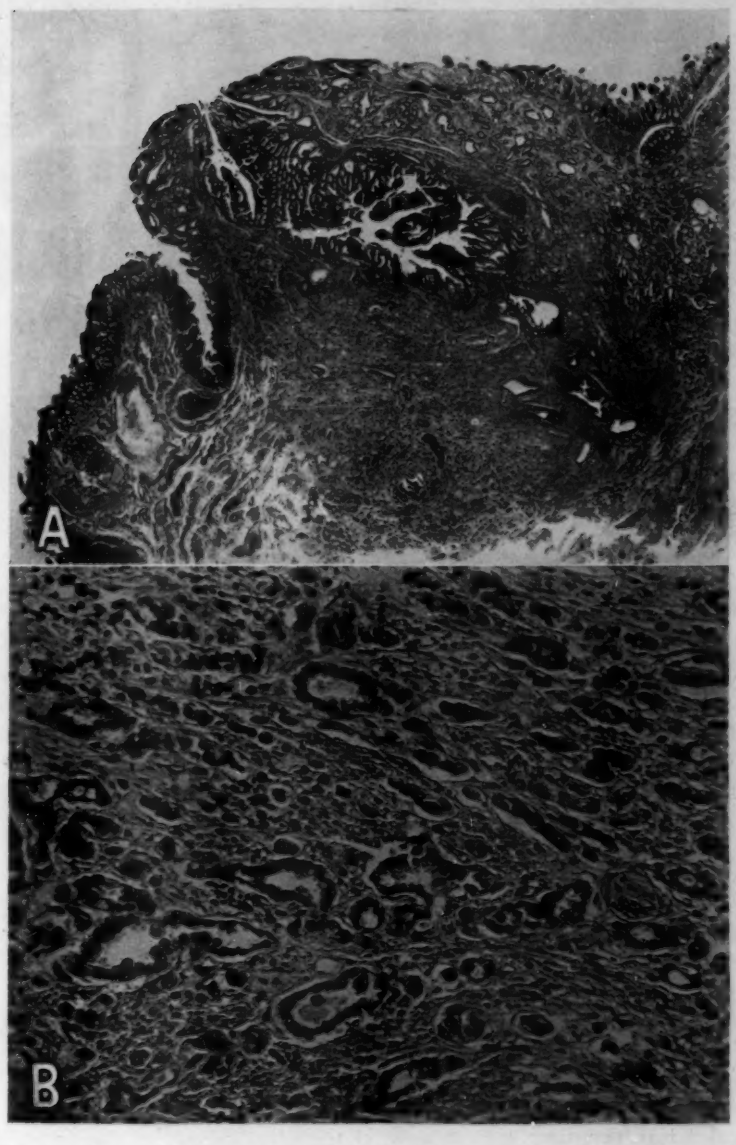

Fig. 1.—*A* is a low power view of a section through the ampulla of Vater to show the limited distribution of the carcinomatous invasion about the termination of the pancreatic duct. *B* is a high power view of a section through the region of the carcinomatous invasion. The tumor is an adenocarcinoma which has stimulated considerable connective tissue growth.

in the head of the pancreas, very close to the termination of the main pancreatic duct, and it had not infiltrated widely (see gross description). It had stimulated considerable local growth of connective tissue (fig. 1*B*).

2. Outside of the small part of the pancreas which contained the adenocarcinoma described, numerous sections from the pancreas showed almost complete disappearance of the acinar tissue (fig. 2). Only a few tiny foci could be found in which some epithelium, greatly altered, was present; these parenchymal remnants represented the remains of a few acini. Some large ducts remained; only a few of the terminal small ducts were seen, and these, together with the

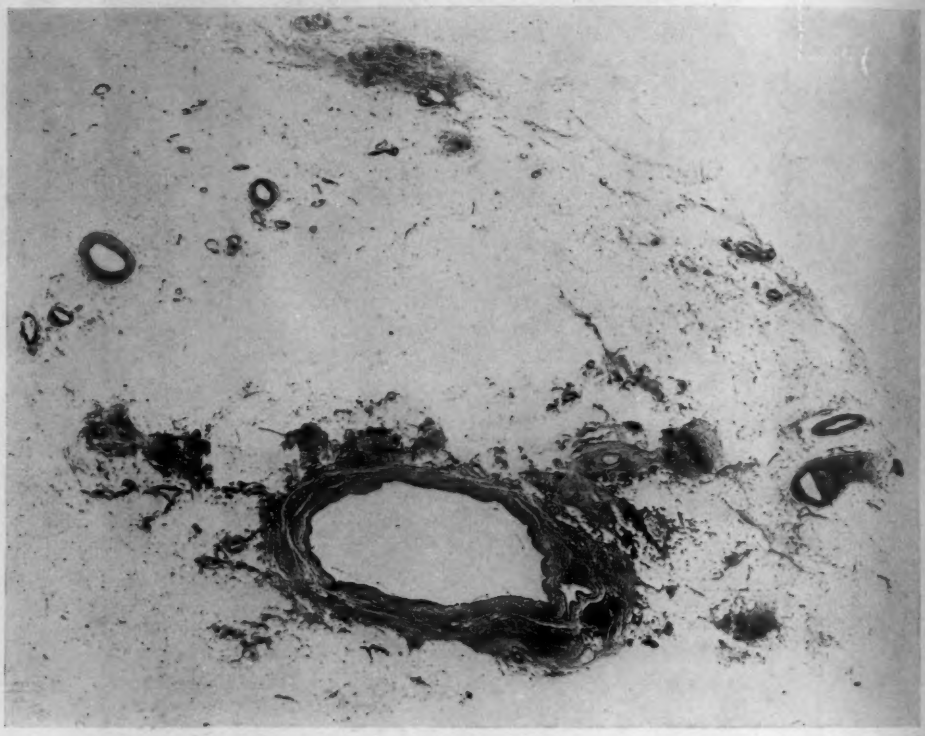

Fig. 2.—This is a very low power view of a section through the pancreas. At the bottom of the figure is seen the greatly dilated pancreatic duct. The denser areas represent islet tissue. The lighter areas represent adipose tissue which has replaced the acinar parenchyma of the pancreas.

sparse acinar remnants, were grouped closely about islands of Langerhans (fig. 3*A*). In a few places some lymphocytic infiltration was noted, but there was no other sign suggestive of an inflammatory reaction. The islands of Langerhans were present in what appeared to be nearly normal numbers, and their structure was normal (fig. 3*A*). There did not appear to be any actual increase in fibrous tissue; such fibrous septums as were present were relatively prominent because of the disappearance of the parenchyma. The parenchyma had been replaced by adipose tissue (fig. 2).

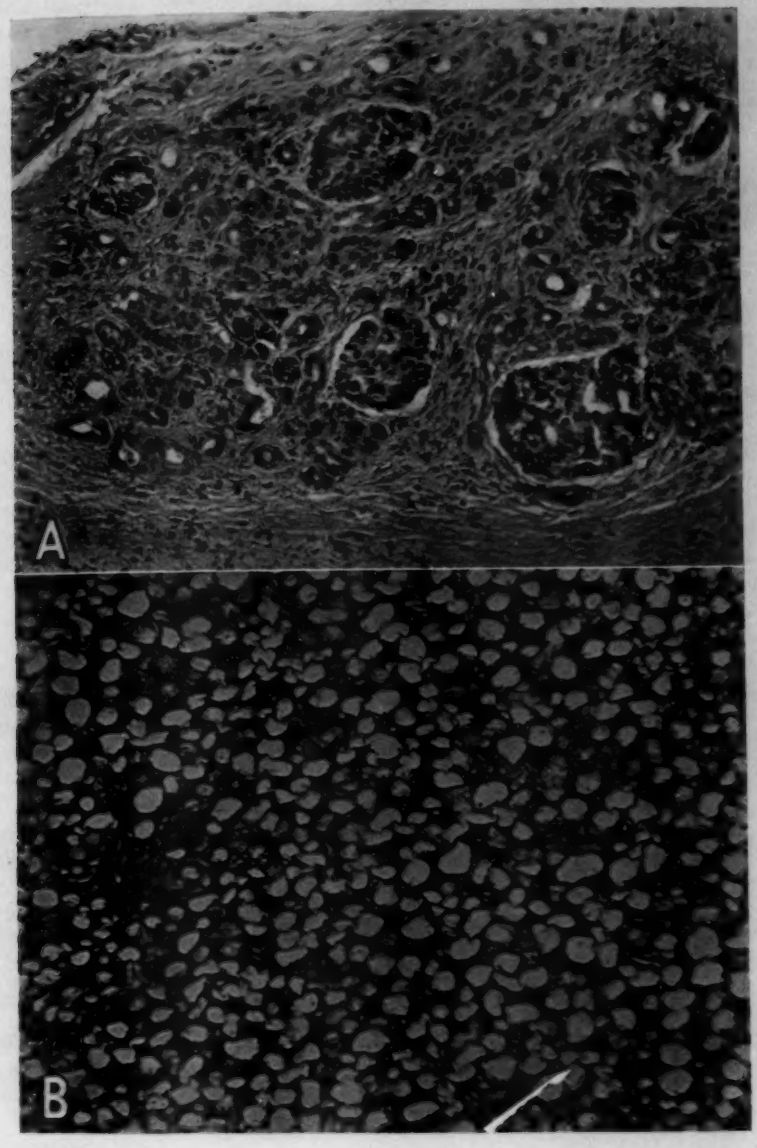

Fig. 3.—*A* is a high power view of one of the denser areas shown in figure 2. Note that the islands of Langerhans are relatively more numerous than in the normal organ and that they appear to be in a good state of preservation. This figure also shows a field which had a maximal amount of acinar tissue remaining. *B* is a median power view of a typical section of liver. Note the extreme degree of fatty metamorphosis and the diffuse involvement of the hepatic lobules by this change.

The liver, in numerous blocks taken from various parts of the organ, showed identical histologic pictures. In paraffin sections stained with hematoxylin and eosin practically every hepatic cell contained a large vacuole, which was surrounded by a narrow zone of foamy cytoplasm or by the cell membrane alone (fig. 3 *B*). The distribution of this vacuolation was uniform throughout the lobules, being no more marked toward the central veins than toward the peripheral parts and the portal spaces (fig. 3 *B*). In frozen sections stained with sudan III and nile blue sulfate the hepatic cells were shown to be nearly filled with neutral fat. A considerable accumulation of granular bile pigment was noted in the narrow cytoplasmic zones of many hepatic cells and in the bile capillaries; some bile pigment was also found in the reticuloendothelial cells lining the sinusoids. There was no evidence of acute or chronic inflammation in the liver and no increase of the fibrous tissue.

The esophagus, stomach (cardia, corpus and antrum), duodenum, jejunum, ileum and colon showed no microscopic evidence of disease.

In the heart there was a markedly increased amount of lipochrome pigment in the cardiac muscle; otherwise the latter appeared normal.

With the exception of very slight arteriosclerotic renal disease, the kidneys showed no microscopic change from the normal. There was no increased amount of fat or lipoids in the renal epithelium. The ureters and the urinary bladder were normal.

Except that the trabeculae were closer together than in the normal spleen, the structural picture in the spleen was normal.

No follicles were present in the ovaries; the stroma was fibrous and contained many corpora albicantia. In the right ovary there were several simple follicular cysts. The fallopian tubes were normal.

In the aorta there was a moderate degree of atherosclerosis without calcification.

The thyroid was made up of follicles, which were lined with flat epithelium, and the follicles were well filled with colloid. The picture was that of a normal quiescent gland.

The two parathyroids examined showed much the same structure so far as the epithelial elements were concerned. The parenchyma was made up of approximately 15 per cent clear chief cells, 75 per cent small vesicular cells, 1 per cent large vesicular cells, 3 per cent scattered individual large pale oxyphil cells and 6 per cent large pale oxyphil cells in islands. This histologic picture is indicative of a moderate degree of secondary parathyroid hyperplasia. The larger glandule contained about 50 per cent adipose tissue and the smaller glandule about 20 per cent.

Lymph nodes from the mediastinum and the mesentery appeared normal.

No gross or microscopic evidence of malignant change was found in any organ except that described in the head of the pancreas.

REVIEW OF THE LITERATURE

Although the case we report is the first human case to be studied in the light of recent experimental researches, the literature contains a few records which need to be cited.

Clarke and Hadfield [1] reported a case under the title of "Congenital Pancreatic Disease with Infantilism": A girl who died at the age of 4 years had suffered from fatty diarrhea certainly from the fifth month of life and had, in all prob-

1. Clarke, C., and Hadfield, G.: Quart. J. Med. **17**:358, 1924.

ability, from birth passed stools containing an excess of fat. The stools averaged five per day, were unformed, voluminous and pale; drops of fat were visible to the naked eye, and microscopically there were many fat droplets but no fatty acid crystals. No improvement took place on a strictly fat-free diet, and death ensued from bronchopneumonia following nasal diphtheria. At postmortem examination the pancreas was smaller than normal, had lost its lobular structure and appeared as a fibrotic, fatty mass. Histologically it was estimated that not more than a twentieth of the normal pancreatic parenchyma remained; the parenchyma had been replaced by fibrous and adipose tissue. There was no evidence of inflammation. The islands of Langerhans were relatively increased in number. The liver weighed 1,247 Gm. and had a uniformly lemon yellow color. Microscopically, each hepatic lobule was represented by a mass of closely packed fat globules. This fatty metamorphosis of the liver was unaccompanied by any sign of necrosis, cirrhosis or inflammation.

Burghard [2] reported the case of a 10 month old child who had suffered from diarrhea since birth with the passage of many putrid fatty stools. At autopsy there was cystic degeneration of the pancreas, with extensive atrophy of the parenchyma. The liver showed fatty degeneration of the periphery of the lobules.

Siwe [3] reported the case of a 2 year old girl, whose symptoms had appeared in the first months of life. She had abnormally frequent large fatty stools. At autopsy nearly total absence of the acinar tissue of the pancreas was found. The acinar tissue had been replaced by adipose tissue. The liver showed a high degree of fatty metamorphosis. This case is thoroughly described and well illustrated, but the author's opinion that it is one of agenesis of the acinar tissue of the pancreas seems to be inadequately supported. As the island tissue was anatomically and functionally normal, it seems likely that the lesion represents early atrophy of the acinar tissue.

Parmelee [4] reported the case of a girl who died at 4½ years of age and who had suffered from steatorrhea since birth. At autopsy, "the pancreas was a hard mass of light gray glistening tissue with a few disseminated, irregular, light grayish-yellow spots. There was no typical pancreatic tissue except at the head, in a region about 6 cm. long, in which there were some lobules. The pancreatic duct at the head of the pancreas was very narrow." Microscopic examination showed nearly complete disappearance of the acinar tissue from the pancreas and marked fatty metamorphosis of the liver.

Garsche [5] reported 2 cases: 1. A boy of 5½ years had, among other symptoms, steatorrhea. At autopsy most of the pancreatic parenchyma had been replaced by fibrous tissue, and the liver showed diffuse fatty metamorphosis. 2. A girl of 4 years had suffered from steatorrhea for two years. At autopsy cirrhosis of the pancreas was found, and the liver showed diffuse fatty metamorphosis.

Fanconi, Uehlinger and Knauer [6] reported the case of a 4½ month old child who presented the complete clinical picture of celiac disease. At autopsy the pancreas showed fibrosis and cyst formation; the liver and cardiac muscle, fatty metamorphosis.

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These 7 cases [6a] are the only ones that we have been able to discover by a review of the literature in which the observations simulated those of our case in their clinical and pathologic aspects. It is interesting that these cases recorded in the literature were all observed in children. There are, however, other reported cases of sprue, celic disease, steatorrhea, pancreatic lithiasis, cystic disease of the pancreas, fibrosis of the pancreas and occlusion of the pancreatic duct by the scar of a duodenal ulcer in which no clear relationship between pancreatic disease and a fatty condition of the liver has been demonstrated.[7]

COMMENT

Ever since the discovery by von Mering and Minkowsky [8] that depancreatization produces hyperglycemia and glycosuria in the dog, reports have appeared in the literature which have tended to implicate the external secretion of the pancreas in the development of diabetes in man (Boldyreff [9]). Much experimental evidence has accumulated, however, to indicate that so far as the pancreas is concerned a condition resembling diabetes develops only when the pancreatic islands are absent or markedly reduced in number. Harms, Van Prohaska and Dragstedt [10] prepared total external pancreatic fistulas in dogs and failed to cause hyperglycemia or glycosuria in their animals. These same workers found that oral administration of activated fresh pancreatice juice to completely depancreatized dogs did not lessen the severity of the diabetes. It thus seems to be conclusively established from experimental studies that there is nothing in the external secretion of the pancreas the absence of which has to do with the development of hyperglycemia or glycosuria.

The case we present strikingly supports the conclusions of Harms, Van Prohaska and Dragstedt.[10] In the first place, no glycosuria was present. In the second place, practically all of the acinar elements of the pancreas were destroyed, and no external secretion of the gland was formed or delivered into the intestine. In the third place, the islands of Langerhans were present in normal numbers and in normal histologic condition.

6a. Since this paper was accepted for publication the report of an eighth case has appeared (Davie, T. B.: J. Path. & Bact. **46**:473, 1938).

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9. Boldyreff, W. N.: Am. J. Digest. Dis. & Nutrition **1**:453, 1934.

10. Harms, H. P.; Van Prohaska, J., and Dragstedt, L. R.: Am. J. Physiol. **117**:160, 1936.

Fisher,[11] Allen and his associates [12] and Best and his associates [13] have shown that completely depancreatized dogs adequately treated with insulin usually fail to survive more than two or three months. The most striking change observed in such animals at autopsy is the extreme fatty metamorphosis of the liver. Allen and his co-workers [12] found that the addition of raw pancreas to the diet of depancreatized animals prevented the development of the hepatic changes and permitted survival for long periods of time. This work has been abundantly confirmed. Berg and Zucker [14] made pancreatic fistulas and ligated the pancreatic ducts; changes in the liver resulted which were similar to those that follow depancreatization, and they concluded that the hepatic effect was probably ascribable to the absence from the intestine of the external secretion of the pancreas. On the contrary, Van Prohaska, Dragstedt and Harms [15] were unable to produce fatty liver (except as might be ascribable to infection) by producing total pancreatic fistula or by ligation of the pancreatic ducts. These investigators were unable to prevent fatty changes in the livers of depancreatized animals by the administration of pancreatic juice, but they were able, like other workers, to prevent this hepatic change by the addition of raw pancreas to the diet. In 1936 Dragstedt and his associates [16] showed that alcoholic extracts of pancreas were effective in protecting the liver of the depancreatized dog against fatty metamorphosis. They concluded that a hormone in the pancreatic tissue was responsible. Kaplan and Chaikoff [17] confirmed the findings of earlier workers regarding the protective effect of raw pancreas and extracts of pancreas in preventing or curing the fatty metamorphosis of the liver which follows depancreatization. They differed, however, from Dragstedt and his co-workers [16] in their opinion regarding the physical qualities of the protective substance and pointed out that, whereas fatty liver may appear as early as three and a half weeks after depancreatization, at least sixteen weeks is needed to insure the development of severe degrees of fatty metamorphosis.

All of these studies have direct bearing on the observations in our case. The changes in the pancreas in our case were such as would result from the ligation of the pancreatic ducts. As previously described and as illustrated in figure 1 *A*, carcinoma chanced to be so situated as to obstruct the pancreatic ducts near their opening into the intestine. Almost complete atrophy of the pancreatic parenchyma resulted. Such

11. Fisher, N. F.: Am. J. Physiol. **67**:634, 1934.

12. Allen, F. N.; Bowie, J. J.; Macleod, J. J. R., and Robinson, W. L.: Brit. J. Exper. Path. **5**:75, 1924.

13. Best, C. H., and Huntsman, M. E.: J. Physiol. **75**:405, 1932. Best, C. H., and Hershey, J. M.: ibid. **75**:49, 1932. Best, C. H.; Ferguson, G. C., and Hershey, J. M.: ibid. **79**:94, 1933. MacLean, D. L., and Best, C. H.: Brit. J. Exper. Path. **15**:193, 1934.

14. Berg, B. N., and Zucker, T. E.: Proc. Soc. Exper. Biol. & Med. **29**: 68, 1931.

15. Van Prohaska, J.; Dragstedt, L. R., and Harms, H. P.: Am. J. Physiol. **117**:166, 1936.

16. Dragstedt, L. R.; Van Prohaska, J., and Harms, H. P.: Am. J. Physiol. **117**:175, 1936.

17. Kaplan, A., and Chaikoff, I. L.: J. Biol. Chem. **119**:435, 1937.

acini as persisted were extremely few and were those grouped closely about the islands of Langerhans. Certainly such acinar tissue as was present constituted far less than 1 per cent of the normal amount of pancreatic parenchyma. Our impression is strong that the islands of Langerhans had not suffered—apparently they were not reduced in number, diminished in size or altered in their structure. They appeared to be relatively increased in number only because of the disappearance of the intervening parenchymal elements. The observations in our case do not seem to agree with the statement of Van Prohaska, Dragstedt and Harms: "These observations suggest that it may not be the loss of acinar tissue which causes the fatty changes in the liver after pancreatectomy, but that this is due to a deficiency in islet function." It seems much more likely to us that, whatever the substance may be, its origin should be sought in the acinar parenchyma of the pancreas. It should further be pointed out that if there was any functional insufficiency of the islands it was not enough to cause glycosuria.

To turn now to the hepatic changes, the fatty metamorphosis of the liver in our case was a diffuse involvement of extreme degree and similar in all respects to the changes found in the livers of depancreatized animals maintained on insulin. The symptoms of pancreatic insufficiency in our case extended over a period of eight months. This is longer than the depancreatized dogs usually survive and longer than Kaplan and Chaikoff found necessary for the development of extreme degrees of fatty metamorphosis in the liver. It should be emphasized that the effects in our case may not have been entirely comparable to experimental depancreatization. In the first place, pancreatic insufficiency in our case may have been enough to account for the steatorrhea at an early date, but the parenchymal atrophy may have progressed slowly. This might be expected to result in protection of the liver for a time. Again it is possible, though less likely, that the obstruction of the pancreatic ducts may have been incomplete or intermittent for a part of the time.

Finally, it needs not be pointed out that our patient died with a condition diagnosed as sprue. The case had been carefully studied, and there were no observations post mortem that could be used to overthrow the clinical diagnosis. The reasonableness of distinguishing between sprue and pancreatic steatorrhea seems to become less clear. We believe that this case should be regarded as one of sprue, and we feel that a consideration of the features which we have presented may suggest an approach to the study of sprue and of spruelike diseases which may lead to clearer notions of their causes.

SUMMARY

We have presented the case of a woman who exhibited the clinical picture of sprue. At autopsy the important pathologic observations consisted of a tiny carcinoma in the region of the ampulla of Vater which had blocked the pancreatic ducts and caused secondary atrophy of the acinar parenchyma of the pancreas from which had resulted fatty metamorphosis of the liver. These anatomic changes are correlated with those produced experimentally by depancreatization of animals. Only 7 other cases (all in infants or young children) with comparable features were found recorded in the literature.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Thomas Francis Jr., a member of the staff of the International Health Division of the Rockefeller Foundation, has been appointed professor of bacteriology in the New York University College of Medicine.

Richard P. Strong, professor of tropical medicine in Harvard Universiy Medical School since 1913, has retired with the title emeritus.

Society News.—The next meeting of the International Congress of Physiologists will be held in England (London, Oxford or Cambridge) in 1941.

The fourth International Congress of Comparative Pathology will be held in Rome, May 15 to 20, 1939. The address of the secretariat of the congress is Consiglio delle Rierche, Piazzale delle Scienze, Rome, Italy.

The next meeting of the American Association of Pathologists and Bacteriologists will be held in the Medical College of Virginia, Richmond, April 6 and 7, 1939.

The first annual meeting of the American Medicolegal Association will be held in the Drake Hotel, Chicago, May 12 and 13, 1939.

The next International Congress for Experimental Cytology will be held in Stockholm, Sweden, in August 1940.

Fellowships.—Fellowships in the medical sciences, administered by the Medical Fellowship Board of the National Research Council, of which Dr. Francis G. Blake, of Yale University, is chairman, will be available for the year beginning July 1, 1939. These fellowships are open to citizens of the United States and Canada who possess the degree of Doctor of Medicine or that of Doctor of Philosophy. They are intended for recent graduates and not for persons already established professionally. Fellows will be appointed at a meeting of the board about March 1. Applications to receive consideration at this meeting must be filed on or before January 1. Appointments may begin on any date determined by the board. For further particulars, address the Secretary of the Medical Fellowship Board, National Research Council, 2101 Constitution Avenue, Washington, D. C.

The Finney-Howell Research Foundation announces that applications for fellowships in cancer research must be sent in by Jan. 1, 1939. Appointments will be made the following March. The secretary is Dr. William A. Fischer, 1211 Cathedral Street, Baltimore.

Abstracts from Current Literature

To Save Space The Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Experimental Peptic Ulcer Produced by Cinchophen. J. L. Bollman, L. K. Stalker and F. C. Mann, Arch. Int. Med. **61**:119, 1938.

Continued administration of cinchophen to a dog produces a chronic gastric ulcer similar in appearance and situation to the gastric ulcer of man. The formation of this ulcer is preceded by acute gastritis, which involves particularly the fundic portion of the stomach. After the first week or two the gastritis is less marked, and a perforating peptic ulcer develops on the pylorus. Within a period as short as three weeks the ulcer may assume all the appearance of a chronic peptic ulcer. Coarse foods decrease and soft foods increase the time required for the formation of the ulcer. During the period of formation the acidity of the gastric juice is within normal limits, but the amount is definitely increased, and the gastric content remains acid longer than normal. Spontaneous healing of the chronic ulcer produced by cinchophen occurs rapidly when the drug is discontinued. Complete healing may be accomplished in from two to seven weeks. In the authors' experiments chronic ulcer did not occur after gastroenterostomy under conditions which produced chronic ulcer in every one of the control animals. Chronic ulcers were not produced in dogs which received a diet of milk and alkaline powders.

From Authors' Summary.

Histologic Changes in the Brain in Experimental Hyperinsulinism. A. Weil, E. Liebert and G. Heilbrunn, Arch. Neurol. & Psychiat. **39**:467, 1938.

The effect of hyperinsulinism on the central nervous system has been studied by Weil and his associates on rabbits by injecting either gradually increasing doses of insulin or large, convulsion-producing doses daily. Some rabbits died suddenly after from twenty-one to twenty-four days of treatment and others after forty-six and fifty-seven days. One rabbit died five hours after a seizure. Widespread changes in the ganglion cells were found—liquefaction, vacuolation and homogenization—and were essentially similar to those described in human beings who have died in hypoglycemic shock, but no meningeal or cerebral hemorrhages were observed. In animals which survived as long as eighty days the ganglion cell changes were mainly a shrinkage of the cell body or of its nucleus. Small doses, about 60 units, produced no changes of the type which were noticeable after injections of from 200 to 400 units. The authors do not think that a vascular factor is responsible for the changes. These were due, in their opinion, to intracellular anoxemia produced by the insulin.

G. B. Hassin.

Experimental "Encephalitis" Produced by Intravenous Injection of Various Coagulants. P. F. A. Hoefer, T. J. Putnam and M. G. Gray, Arch. Neurol. & Psychiat. **39**:799, 1938.

Hoefer and his co-workers produced thrombi in various organs, including the brains of dogs, rabbits and cats by injecting intravenously various coagulants, such as homologous or heterologous serum and extracts of lung or brain. In addition, they produced in some animals cerebral lesions resembling those seen

in postvaccinal encephalomyelitis. The histologic changes varied according to the dose injected and were seen either as proliferation of oligodendroglia and formation of perivenous cuffs, composed chiefly of microglia cells, mixed with a few cells resembling lymphocytes and plasma cells, or as proliferation of astrocytes or anemic ganglion cell changes in the cortex, with satellitosis. In some animals there was only congestion of the veins, without visible parenchymatous changes. In other animals no demonstrable lesions in the nervous system were present.

G. B. HASSIN.

BLOOD PLASMA PROTEIN REGENERATION. S. C. MADDEN and others, J. Exper. Med. **67**:675, 1938.

When the proteins in the blood plasma have been depleted by plasmapheresis (bleeding with return of the washed red cells) it is possible to bring about a steady state of hypoproteinemia and a uniform production of plasma protein on a basal diet low in protein. The dogs are clinically normal, with normal appetite; they do not have anemia, and their metabolism of nitrogen is normal. Such dogs become proper subjects in which to investigate various factors in the production of plasma protein. The normal dog (weighing from 10 to 13 Kg.) has a substantial reserve of plasma protein building material (from 10 to 60 + Gm.), which can be completely removed by from two to six weeks of plasmapheresis. After this period the dog will produce uniform amounts of plasma protein each week on a fixed basal diet. Dogs depleted by plasmapheresis and then permitted to return to normal during a rest period of many weeks may show much higher reserves of protein building material in subsequent periods of plasma depletion. Under uniform conditions of low intake of protein in the diet, when plasmapheresis is discontinued for two weeks the plasma protein building material is stored quantitatively in the body and can be recovered in the next two to three weeks of plasmapheresis. Given complete depletion of the stores for building plasma protein, the dog can still produce a little plasma protein (2± Gm. per week) on a protein-free diet. This may be related to the wear and tear of body protein and conservation of the split products. Abscesses produced in a depleted dog during a fast may be associated with an excess of plasma protein, which is probably related to products of tissue destruction conserved for protein anabolism. Gelatin alone added to the basal diet causes little production of plasma protein but when supplemented by tryptophan gives a large output of protein, though tryptophan alone is inert.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL SPLENOMEGALY. T. B. MENON, J. Path. & Bact. **46**:357, 1938.

In the livers of rats and rabbits portal obstruction has been induced by various operative measures having the effect of narrowing the lumen of the portal vein After complete occlusion of the vein there is splenic enlargement of twice or three times the normal and no more; the condition of the spleen is one of venous infarction. After partial obstruction for from three to six months there is little enlargement of the spleen. The spleen shows dilatation of the sinuses, distention of the pulp and trabecular veins, and variable atrophy of the pulp, with slight fibrillary increase. Hyperplastic reactions are absent. These experiments do not support but seem to speak against the view that the Banti-syndrome is due to blockage of the portal vein.

FROM AUTHOR'S SUMMARY.

RETARDING ACTION OF ETHYL ESTERS OF SATURATED FATTY ACIDS ON EXPERIMENTAL TUBERCULOSIS OF THE GUINEA PIG. L. NÈGRE, A. BERTHELOT and J. BRETEY, Ann. Inst. Pasteur **59**:457, 1937.

Ethyl esters of saturated fatty acids of relatively high molecular weights (palmitate, laurate, stearate, myristate, caprate and arachidate) retard the development of lesions in tuberculous guinea pigs when injected subcutaneously in doses of from 0.2 to 0.5 cc. biweekly.

Ethyl caprylate and caproate do not retard the development of lesions. Ethyl butyrate activates the formation of tuberculous processes.

Ethyl palmitate showed the most marked and most consistent retardative action. Its effect was enhanced by the addition of methyl antigen.

When the active saturated fatty acids are combined with other alcohols, especially benzylic and cinnamic, the results are negative.

Prophylactic injections of large doses of ethyl palmitate, laurate and stearate do not seem to modify the tuberculous processes of guinea pigs subsequently infected, as they do when they are used therapeutically. Tuberculous guinea pigs treated with the most active fatty acids show marked hypertrophy of the spleen, in which one often finds no lesions. Histologic examination of this organ shows reticular hyperplasia with a collagenous appearance. The lungs show thickening and infiltration of the stroma. The lymph nodes show more sclerosis than those of untreated animals.

FROM AUTHORS' CONCLUSIONS.

EXPERIMENTAL SCURVY OF THE GUINEA PIG. M. GLASUNOW, Virchows Arch. f. path. Anat. **299**:120, 1937.

This lengthy contribution is divided into three parts: The first deals with age as a factor in the development of experimental scurvy in the guinea pig. The characteristic triad of changes in the teeth, abnormalities of bones and tendency toward hemorrhage occurs only in the young, growing animal. The scorbutic process appears to have a relation to the growth energy of the animal. In the adult guinea pig dental changes are most uniformly observed. In the guinea pig the teeth continue to grow throughout life. After bone growth has ceased, osteoporosis is usually the only change noted in the bones. The tendency to bleed may be very slight or entirely absent. The experimental scurvy of the adult guinea pig sheds light on some of the human osteopathies.

The second part of the paper presents the results of a study of the healing of the dental and osseous lesions. Under treatment these may heal so rapidly that all evidence of their occurrence may disappear within from thirty to forty days. New normal dentin is formed; the pathologic dentin is not organized. In the bones periosteal and endochondral ossification again become evident; callus forms at the sites of fracture; normal marrow is restored in the spongy bone, and abnormal and excessive bone is removed. The rapidity and completeness of the disappearance of the osseous defects bear a direct relationship to the dose of vitamin C.

The third section deals with the structural changes in the zone of endochondral bone growth. Study of the costal rosary of experimental scurvy leads to acceptance of the view that the cells of Ranvier's groove act as a cambium at the expense of which longitudinal growth of the costal cartilages occurs. The fibrous marrow forms an internal or endosteal callus which undergoes no further differentiation. The osteoporosis of scurvy is the result of fibrous atrophy of bone. Scurvy is a disease of the entire mesenchyme, characterized by a loss of the property of differentiation on the part of the newly formed mesenchyme. The mode of action of vitamin C is unknown.

O. T. SCHULTZ.

Pathologic Anatomy

HYPERTENSIVE APOPLEXY AND ITS CAUSATION. W. H. CHASE, Arch. Neurol. & Pychiat. **38**:1176, 1937.

Hypertension may occur in the absence of arteriosclerosis and may be so-called essential hypertension or uncomplicated hypertension exhibiting arterial changes which differ from those seen in arteriosclerosis. These types may be associated with changes in the brain and kidneys. Of 108 cases of cerebral hemorrhage there were only 18 that were uncomplicated; in some the lesions were of a few weeks' or months' duration; in others they had lasted two years. The arterial changes in cases of long-continued essential hypertension were (1) regular, uniform

thickening of the intracranial muscular arteries, due to compensatory muscular hypertrophy, (2) a widening of the arterial lumen and (3) the presence of blood clots, their centers consisting of laminated fibrin, blood cells and many threadlike blood vessels. The perivascular clots appeared as protrusions which did not originate from the lumen (pseudoaneurysms). The punctate hemorrhages were multiple and occurred in the corpus striatum, optic thalamus and pons. In transient hypertension, such as that seen in patients with eclampsia, no macroscopic hemorrhages in the brain were demonstrable. The arteries were not thickened, pseudoaneurysms were absent, and muscular hypertrophy was not in evidence. Chase sees the cause of hemorrhages in paralysis of the small blood vessels (paretic vasodilatation), which may be preceded by vascular spasm, and he thinks that even large hemorrhages may occur in the brain "by diapedesis from terminal districts and from the vasa vasorum of hypertrophied paretic muscular arteries."

GEORGE B. HASSIN.

ENOSTOSES OF THE CALVARIUM. M. M. CANAVAN, Arch. Neurol. & Psychiat. **39**:41, 1938.

Enostosis is the name given by Canavan to a morbid manifestation generally known as hyperostosis, endostosis, osteophyte, osteoma, osteitis or exostosis. It is an irregular growth of dense bone tissue, with the diploe transformed into tissue of ivory-like firmness, protruding from the inner table of the bone into the cranial cavity and pressing on the dura, to which it is adherent. The dura here is often absorbed, appearing ribbon-like or shredded. Canavan studied the necropsy records of 3,250 persons with lesions of this type, 230 (7 per cent) of whom exhibited an enostosis of the calvarium. The sex, age and condition of the blood vessels and internal organs (liver, kidneys) did not have any causal relationship to the bone anomaly. The pituitary exhibited no particular changes. Enostosis occurred in association with every type of psychosis and was particularly frequent in association with senile dementia and dementia praecox. The duration of a psychosis had no relationship to the frequency of its occurrence. In 3.86 per cent of cases of enostosis, an intracranial tumor was found—glioma or meningioma.

GEORGE B. HASSIN.

LYMPHOGRANULOMATOUS SALPINGITIS. R. D'AUNOY and J. R. SCHENKEN, J. A. M. A. **110**:799, 1938.

In a case of salpingitis with microscopic lesions consistent with those found in other organs during the course of venereal lymphogranuloma the patient gave a positive reaction to the Frei test and gave no other evidence of being or having been infected with the virus of venereal lymphogranuloma. The case shows the necessity for considering venereal lymphogranuloma as an etiologic factor in pelvic inflammatory disease, particularly in the Negro race. FROM AUTHORS' SUMMARY.

HISTOLOGIC CRITERION FOR DIFFERENTIAL DIAGNOSIS OF YELLOW FEVER. J. BABLET and F. BLOCH, Ann. Inst. Pasteur **59**:492, 1937.

A histologic examination of the lesions in the liver is of diagnostic value in yellow fever. Frozen sections stained with hemalum–scarlet red are examined for evidence of fatty degeneration. The absence of this change rules out yellow fever. If fatty degeneration is present, the tissue is fixed in alcoholic trinitrophenol-formaldehyde solution and paraffin sections are stained with hemalum-eosin-safranin or with Mallory's phosphotungstic acid–hematoxylin. In yellow fever hyaline necrosis, characterized by nuclear chromatolysis, is found. There are typical areas of necrotic cells devoid of nuclear structure scattered among less numerous normal cells.

Other changes in the liver are not of differential value because of individual variation. Lesions of the kidney, myocardium and spleen cannot be used as diagnostic criteria.

The intranuclear inclusions of Torres, demonstrated by Mann's method, are of differential value, but the technic is delicate and exacting.

FROM AUTHORS' CONCLUSIONS.

ATYPICAL HODGKIN'S DISEASE. M. MITTELBACH-SCHMIDT and W. STOLZ, Frankfurt. Ztschr. f. Path. **50**:365, 1937.

Mittelbach-Schmidt and Stolz present 2 cases of Hodgkin's disease in which the lesions were atypical. In the first, Hodgkin's granulomas were found in the renal glomeruli of a 45 year old woman. Microscopically the granulation tissue contained many mononucleated giant cells. The lymph nodes, liver, myocardium and bone marrow were also involved. In the second instance, that of a 34 year old woman, the outstanding observation clinically and pathologically was a markedly enlarged spleen, weighing 4,050 Gm. and measuring 36 by 24 by 12 cm. Areas of necrosis were abundant, and there was marked proliferation of reticuloendothelial cells, while no multinucleated Dorothy Reed cells were observed. Similar reticulum cells were present in the periaortic, portal and peripancreatic lymph nodes and in the liver and bone marrow.

ANNEMARIE STRAUSS.

GENESIS OF MULTIPLE CYSTS OF THE LUNG. B. ROMBANYI and B. MACCONE, Frankfurt. Ztschr. f. Path. **50**:442, 1937.

Polycystic lungs in embryos or newborn infants are normally explained on the basis of congenital bronchiectasis. In adults, however, a congenital character of these cysts is not always traceable. The authors present a 16 year old boy who suffered from severe cyanosis for one year. At autopsy multiple cystlike structures were distributed throughout both lungs and were filled with air. After careful examination the authors concluded that the cysts developed from dilated respiratory bronchioli. Dilated bronchioli were also observed in places where no definite cysts were seen. This indicated to the authors the progressive nature of the cyst formation. Sometimes normal alveoli were attached to the cysts. The authors feel that the chronic peribronchiolitic changes which were found on microscopic examination and which probably followed measles were the basis for the dilatation of the bronchioli.

ANNEMARIE STRAUSS.

OBLITERATING ENDOPHLEBITIS OF THE HEPATIC VEIN. C. CORONINI and G. OBERSON, Virchows Arch. f. path. Anat. **298**:250, 1936.

Eleven cases form the basis of this histologic study. The process described involves the central vein of the hepatic lobule. Degenerative and inflammatory changes in the intima and necrosis of the media may lead to obliteration of the vessel, and to central cirrhosis. The process does not involve the adventitia. It may spread to the intralobular capillaries. These may at times be involved when the central vein escapes. Occasionally the interlobular branches of the portal vein may be similarly involved. Similar changes in the veins of the spleen may lead to the condition described by Banti. The same process occurs less frequently in the kidneys. The earliest stage of the lesion is described as a serous fibrillary inflammatory reaction similar to the hyperergic reaction of rheumatic infection. It is in the category of rheumatic allergic inflammatory reactions that the process described is placed.

O. T. SCHULTZ.

ABSENCE OF THE RIGHT PULMONARY ARTERY. F. BRENNER, Virchows Arch. f. path. Anat. **298**:394, 1936.

Congenital absence of the right pulmonary artery is an extremely rare anomaly, only 2 cases having been previously reported. Absence of the left pulmonary artery is somewhat more frequent. In Brenner's patient death occurred at the age of 8 weeks. The heart was hypertrophied. The root of the pulmonary

artery give rise to the left pulmonary artery and a narrow ductus arteriosus. The right lung was supplied by an artery given off from the upper part of the thoracic aorta. The anomaly is ascribed to abnormality in the development of the sixth branchial artery. O. T. SCHULTZ.

ATYPICAL AMYLOIDOSIS IN A CASE OF MULTIPLE MYELOMA. W. VOLLAND, Virchows Arch. f. path. Anat. **298**:660, 1937.

A case of plasmacytic multiple myeloma with atypical amyloidosis of the heart, lungs, liver and kidneys is made the basis of a consideration of atypical amyloidosis. The latter condition is held to be the result of activation of connective tissue by abnormal derivatives of body protein. The reactivity of the connective tissue is insufficient to denature the protein completely, and the protein is deposited as amyloid or paramyloid. In typical amyloidosis it is the endothelium and reticuloendothelium that are activated. O. T. SCHULTZ.

INTESTINAL FINDINGS IN NONTROPICAL SPRUE. H. ROSENTHAL, Virchows Arch. f. path. Anat. **298**:706, 1937.

Rosenthal reviews the literature relating to the occurrence of sprue in nontropical countries, describes the clinical symptoms, comments on the etiologic factors, which he considers still undetermined, and presents the intestinal findings in 3 persons with this disease who came to necropsy in Hanover, Germany. The process is one of chronic catarrhal inflammation of the small intestine and cecum, with polypoid overgrowth of the mucosa of the jejunum and ileum and small ulcers of the mucosa. One of these persons had a small ulcer which had perforated. The lesions are not characteristic in the gross nor are they histologically specific. But when they are considered in the light of the clinical manifestations, the disease appears to be a clinicopathologic entity. O. T. SCHULTZ.

DIAGNOSTIC IMPORTANCE OF FUNCTIONAL CHANGES IN THE ENDOMETRIUM. H. BANIECKI, Virchows Arch. f. path. Anat. **299**:376, 1937.

The function of the endometrium is held to be the nidation of the fertilized ovum. The criteria of normal functioning are the cyclic changes. The most important morphologic evidence of function is secretory activity of the epithelial cells of the endometrial glands, evidences of which are to be detected throughout the cycle. The secreting cells and their secretion contain glycogen. Other evidences of function are hypertrophy and hyperplasia of the glandular epithelium, with change in the shape and location of the nucleus, hyperplasia of the interglandular stroma and tortuosity of the endometrial vessels. An endometrium that does not reveal the changes described is termed a nonfunctioning endometrium. Endometrium showing glandular hyperplasia and that showing premature atrophy and fibrosis are the most common examples of nonfunctioning endometrium. Pathologic functioning of the ovary leads to delayed casting-off of the endometrium after menstruation, to delayed regeneration after childbirth and abortion and to glandular hyperplasia, conditions in each of which there is morphologic evidence of nonfunction of the endometrium. In the first two the ovarian dysfunction is slight and temporary, and curettage is usually followed by formation of a normal endometrium. Glandular hyperplasia results from death of the ovum and failure of rupture of the follicle, with consequent failure of development of a normal corpus luteum. In young women up to 25 years of age the prognosis of glandular hyperplasia is bad, because the condition is associated with sterility. In women of from 25 to 35 years the condition is less grave, and curettage may be followed by pregnancy. Throughout his lengthy contribution the author has much to say about glandular hyperplasia and its correct diagnosis. What he has to say leaves the reader confused and doubtful whether the distinctions the author makes are warranted. The material studied was obtained by the usual uterine curettage.

The reproduced photomicrographs leave much to be desired in the way of cytologic detail, and it is on the latter that the conclusions are based.

O. T. SCHULTZ.

Microbiology and Parasitology

AVIAN TUBERCULOSIS. A. B. CRAWFORD, Am. Rev. Tuberc. **37**:579, 582, 588 and 594, 1938.

It has been known for a number of years that a great majority of the retentions of swine for tuberculosis in federally inspected abattoirs were because of infection with avian tubercle bacilli, but this type of infection was thought to be nonprogressive and confined chiefly to the lymph nodes of the alimentary tract. In 1936 Feldman found 24 of 30 carcasses of swine that had been condemned because of generalized tuberculosis in an abattoir in Minnesota to be affected with the avian type of infection. This report covers the typing of lesions in 36 swine condemned for tuberculosis in five of the North Central States; 21 of the specimens were found to be affected with the avian type of tubercle bacillus and 15 with the bovine type.

In a series of 42 rabbits inoculated intravenously with suspensions of the avian type of tuberculous tissue from swine and a series of 30 rabbits inoculated with similar suspensions of tissues showing the bovine type of infection from swine, the bovine type of tubercle bacillus was shown to be decidedly more virulent for the rabbit than the avian type. In the latter series, 100 per cent of the rabbits died of tuberculosis within ninety-nine days, whereas in the series inoculated with bacilli of the avian type only 23 per cent died within this period. In the series inoculated with bacilli of the bovine type the lungs were the chief site of localization, while in the other series the lungs were affected relatively slightly and the chief site of localization was variable.

The differentiation of avian from bovine infection by means of sensitization in swine tuberculosis is shown to be specific. Of 36 specimens, 21 caused in guinea pigs a sensitization specific to avian tuberculin, and 15, to mammalian tuberculin. These figures were in complete agreement with those from animal inoculation and culture typing. The results of an experiment are shown which indicate that dual infection with avian and bovine tubercle bacilli may be detected by the sensitization method.

The few proved cases of the avian type of tuberculous infection in man indicate that the human species is resistant to this type of infection or that the amount of exposure is insufficient to cause disease except in rare instances. Persons tending tuberculous flocks suffer the greatest exposure not only by possible ingestion of infected material but also by inhalation. The chief manner in which the general population may be exposed is through the ingestion of eggs from hens affected with tuberculosis of the oviduct. If tuberculosis in poultry were to become so widespread as to increase the frequency of man's exposure, it is conceivable that this type might not be without danger to those who are least resistant. In the light of present evidence, however, it does not seem that the avian type of tuberculosis presents more than a remote possibility of danger to man.

FROM AUTHOR'S SUMMARIES.

RESULTS OF THE INTRATRACHEAL INJECTION OF THE BORDET-GENGOU BACILLUS IN THE MONKEY. D. H. SPRUNT, D. S. MARTIN and S. MCDEARMAN, J. Exper. Med. **67**:309, 1938.

Experiments are reported which show that the virulent Bordet-Gengou bacillus can produce significant lymphocytosis and interstitial mononuclear pneumonia in both the monkey and the rabbit. Both of these reactions occur apparently in response to a toxic material formed in vivo by the Bordet-Gengou bacillus and are not dependent on the multiplication of the organism itself. It was also shown that the strictly avirulent form could also cause interstitial mononuclear pneu-

monia but not lymphocytosis. This interstitial mononuclear pneumonia was thought to be a reaction to the foreign substance produced by the organism when it was in the avirulent stage—an inference which was substantiated by the fact that this lesion could be produced with both living and dead organisms.

FROM AUTHORS' SUMMARY.

CANINE DISTEMPER IN THE RHESUS MONKEY. G. DALLDORF, M. DOUGLASS and H. E. ROBINSON, J. Exper. Med. **67**:323 and 333, 1938.

Canine distemper has been transmitted to rhesus monkeys by a variety of methods. The disease in monkeys is strikingly similar if not identical with the distemper in dogs.

Rhesus monkeys to which canine distemper has been transmitted are relatively or completely immune to experimental poliomyelitis during the first two weeks of the distemper. Monkeys convalescent from the distemper are not resistant to experimental poliomyelitis. Two monkeys vaccinated with the virus of canine distemper responded to poliomyelitis in a modified manner. Distemper antiserum did not influence the course of experimental poliomyelitis in rhesus monkeys. Equine encephalomyelitis and vaccinial encephalitis showed no sparing effect on the course of experimental poliomyelitis. The concurrence of distemper and poliomyelitis in monkeys seems to represent a new immunity mechanism in the field of the viruses.

FROM AUTHORS' SUMMARIES.

CULTIVATION OF THE RICKETTSIAS OF ENDEMIC (MURINE) AND EPIDEMIC (EUROPEAN) TYPHUS FEVER IN VITRO. I. A. BENGTSON, Pub. Health Rep. **52**:1336, 1937.

The rickettsias of endemic typhus fever have been cultivated in vitro through 17 passages. Luxuriant growths were obtained in modified Maitland mediums in which Baker's solution was substituted for Tyrode's solution. The rickettsias of European typhus grew almost as luxuriantly in similar mediums.

FROM AUTHOR'S SUMMARY.

THE HYPOTHERMIC FACTOR OF BACILLUS DYSENTERIAE SHIGA. L. OLITZKI and S. AVINERY, Brit. J. Exper. Path. **18**:316, 1937.

The experiments reported indicate that there exists in Bacillus dysenteriae Shiga, as well as in some other micro-organisms, a fraction which causes a fall in the temperature of the body. In certain cases this drop may be preceded and followed by hyperthermic reactions. This substance has antigenic properties, and it is possible to immunize guinea pigs against it actively by injecting large quantities (from 4 to 5 mg.) of bacteria and passively by previously injecting immune serum. This factor is associated with the protein fractions of the bacteria, mainly with P 1 or, if the products of trypsin digestion are used, with F68/68. The lipoid and carbohydrate fractions produced only a rise in temperature. The effect of P 1 and of F68/68 on the temperature of the body is interesting, because these substances also possess high biologic activity in other directions. According to Olitzki, Leibowitz and Berman, P 1 has relatively low toxicity for rabbits but produces in these animals both hyperglycemia and leukopenia. Olitzki recently also found that it is relatively highly toxic for mice. The fraction F68/68 from Salmonella typhi murium is, according to Raistrick and Topley, a substance of high toxicity and immunizing value. Delafield found that it produces hyperglycemia, and Delafield and Smith reported certain changes in the oxygen uptake of tissues due to it. The two substances P 1 and F68/68 seem, therefore, to be closely related, if not identical. It is at the moment impossible to say what is the mechanism of the hypothermic effect, but it can be stated that younger animals react more readily; adult uninfected animals are less sensitive, and infected animals, in which the regulation of temperature is disturbed, are highly sensitive.

FROM AUTHORS' SUMMARY.

THE NUTRITION OF STAPHYLOCOCCUS AUREUS. G. P. GLADSTONE, Brit. J. Exper. Path. **18**:322, 1937.

Of 26 strains of Staphylococcus aureus, 25 grew well on a medium of known chemical composition which included 16 aminoacids. Initial differences in amino-acid requirements were found among these strains. The organisms were easily adapted to utilize fewer aminoacids; then such differences tended to disappear. Finally, by a process of training, strains were produced which could grow on a medium from which all aminoacids were excluded, in which the main source of nitrogen was ammonia.

FROM AUTHOR'S SUMMARY.

PATHOGENICITY FOR ANIMALS OF VIRUSES FROM HUMAN INFLUENZA. J. MCINTOSH and F. R. SELBIE, Brit. J. Exper. Path. **18**:334, 1937.

Two strains of virus isolated from human lungs in cases of influenza were found capable of causing lesions of the lungs in animals. The lesions were produced in ferrets, mice, rabbits, guinea pigs and a monkey by intranasal inoculation of the virus under anesthesia. Preliminary immunologic tests showed definite protection by convalescent influenza serum and by the anti-influenza serum $1H_2$ prepared by Smith, Andrewes and Laidlaw against their influenza virus.

FROM AUTHORS' SUMMARY.

THE BACILLI OF LEPROSY AND THE NERVOUS SYSTEM. E. MARCHOUX, V. CHORINE and D. KOECHLIN, Ann. Inst. Pasteur **59**:549, 1937.

Rats often withstand Stefansky's bacilli injected into the cranial cavity or even into the cerebral tissue without apparent change in general health or in behavior. There is rapidly progressive invasion of the meninges, especially of the dura mater. The organisms penetrate the cerebrum and cerebellum, spreading into the cortex, choroid plexus and ventricular ependyma.

Intracerebral inoculation calls forth histiocytes, which form a series of nodules along the path of the needle. The organisms spread by the lymph and blood stream and accumulate in the perivascular zones or rarely in the endothelial cells of the blood vessels. Perivasculitis is especially marked in the olfactory lobe and medulla oblongata. The molecular layer of the cerebellum is often destroyed, and the cells of Purkinje lose their prolongations and become rounded. The medulla is infected early, especially the meninges, and the organisms invade the posterior roots, spinal ganglions and cauda equina. The ependymal canal is infected, and histiocytes containing bacilli migrate into the medulla. The organisms are found in the sciatic nerve, especially in Henle's sheath, and in the lymphatic, sciatic and popliteal ganglions. Groups of organisms occur in certain cells of Schwann's sheath.

There is marked infection of the meninges of the optic nerve up to its branching in the retina. When the organisms are inoculated by way of the eye, the infection extends to the cerebral meninges.

The nerve tissue itself shows marked resistance to leprous infection. It is injured by compression due to adventitious cells forming nodules, but the organisms rarely penetrate the nerve cells. Marked cerebral edema is present and may even alter the cells. Experiments on chronaxia indicate an irritability of the nervous system of meningeal origin.

FROM AUTHORS' SUMMARY.

THE NEUROLYMPHOPHILIC VIRUS. M. PETZETAKIS, Zentralbl. f. Bakt. (Abt. 1) **139**:197, 1937.

This paper describes the results from the injection into monkeys, rabbits, guinea pigs, cats, dogs, white rats and mice of filtrates from pus obtained from a patient with subacute monoadenitis. Petzetakis believes that the lymphophilic virus possesses an unmistakable affinity for the nervous system. Direct intracerebral injection called forth typical meningoencephalitis. The most susceptible animals were the marmot (spermophilus), the rabbit and the guinea pig.

PAUL R. CANNON.

BLOOD CHANGES IN EXPERIMENTAL TUBERCULOSIS. K. E. BIRKHAUG and T. GUTHE, Acta tuberc. Scandinav. **11**:1, 1937.

The S variant of the avian type of Mycobacterium tuberculosis is highly virulent for chickens and rabbits in small doses (0.01 mg.) injected intravenously and only relatively virulent for guinea pigs in larger doses (from 1 to 0.1 mg.) injected intradermally. The tuberculous histocytologic activity is faithfully mirrored in the current blood picture in the form of progressive anemia, an increased rate of sedimentation of red blood cells, lymphopenia and monocytosis or in a reversal of the monocyte-lymphocyte ratio. The degree of virulence of the avian S variant for chickens, rabbits and guinea pigs is also reflected in the persistent tuberculous bacillemia in the chicken and rabbit and the transitory bacillemia in the guinea pig.

The R variant of the avian type of M. tuberculosis is relatively avirulent for chickens, rabbits and guinea pigs in large doses injected parenterally, and the benign histocytologic evolution of the avian R variant infection is likewise faithfully mirrored in a transitorily altered blood picture, which rapidly returns to normal limits.

Hypersensitiveness toward standard tuberculin is rapidly and persistently produced in the guinea pig following parenteral injection of both S and R variants of the avian tubercle bacillus. The tuberculin allergy is severest with the S variant infection but persists with both S and R variant infections long after the macroscopic tuberculous lesions are healed. These experiments clearly demonstrate that a close correlation exists between the histocytologic evolution of infections with the S and R variants of the avian type of M. tuberculosis, on one hand, and the changes in the peripheral blood, on the other, and that a direct relation exists between the tuberculous process and the persistent increase in the monocyte-lymphocyte ratio. Occasional fluctuations in the rate of sedimentation in gravely tuberculous animals makes this reaction, taken by itself, less sensitive than the monocyte-lymphocyte index. Combined with the latter, the rate of sedimentation remains a valuable indicator of the constitutional disturbance brought about by tuberculous disease. FROM AUTHORS' SUMMARY.

Immunology

ANAPHYLACTIC SHOCK BY AZODYES. K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. **67**:79, 1938.

From the experiments presented, it follows that the specific precipitation and the production of anaphylactic shock with certain azodyes as described previously are due to these substances themselves and are not dependent on azoproteins formed by interaction of the dyes with proteins in the test tube or in the animal body. Besides these, some other azodyes, which in the tests did not give precipitation with corresponding immune serums, were found, when used in small quantities, to induce anaphylactic contraction of the uterus of the sensitized guinea pig.

FROM AUTHORS' SUMMARY.

ANAPHYLAXIS IN THE ISOLATED HEART. H. B. WILCOX JR. and A. E. COWLES, J. Exper. Med. **67**:169, 1938.

Isolated hearts of guinea pigs sensitized to horse serum have been shown to react characteristically on exposure to small amounts of the antigen. The cardiac rate is temporarily accelerated, and transient alterations occur in the amplitude of contraction. Electrocardiographic abnormalities, previously recorded by remote leads during anaphylactic shock in the intact animal, have been recorded by direct leads from the isolated perfused hearts of sensitized animals during this reaction. An additional effect of anaphylaxis in the isolated heart of the guinea pig is reported: a striking reduction in the rate of flow through the coronary vessels.

The anaphylactic reaction of the isolated heart of the guinea pig has been compared with the action of histamine on the same preparation, and the effect of atropine on each has been observed. The implications of certain quantitative differences in the influence of atropine on these reactions are discussed.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL HYPERSENSITIVENESS IN RHESUS MONKEY. H. W. STRAUS, J. Immunol. **32**:241 and 251, 1937.

An extract of poison ivy applied to the skin of rhesus monkeys did not produce any local change, but when similarly applied again ten days later it produced lesions similar to the dermatitis that follows contact in man. The hypersensitiveness continued for eight months but was weaker after four months. The monkeys reacted also to poison oak. The reactions were similar to those of human infants and different from those of animals which had reacted to the extract of poison ivy when it was first applied. The skin of rhesus monkeys could be passively sensitized with the serums of persons sensitive to horse serum, cottonseed, peanut and flounder. The sensitivity was tested by injecting intracutaneously the homologous antigen into the site where the serum had been injected or by injecting intravenously. Serum with a low titer of reagin failed to sensitize. The local reaction was represented by edema, but as a rule there was no erythema or pruritus. The specific nature was established by proper controls. I. DAVIDSOHN.

SIMULTANEOUS CUTANEOUS AND LYMPHNODAL SHWARTZMAN PHENOMENON. E. E. ECKER, H. T. KARSNER and L. MESCHAN, J. Infect. Dis. **62**:1, 1938.

The intracutaneous injection of the preparatory factor excites inflammation at the site of injection and in the lymph nodes of the local drainage area. The subsequent intravenous administration of the provocative factor provokes that augmentation of inflammation, together with alteration of its character, in both skin and lymph nodes, which is characteristic of the Shwartzman phenomenon.

FROM AUTHORS' SUMMARY.

LOCAL IMMUNITY TO SCARLET FEVER TOXIN. G. F. DICK and G. H. DICK, J. Infect. Dis. **62**:83, 1938.

Local, as distinguished from general, antitoxic immunity to scarlet fever toxin, either partial or complete, may be produced by intradermal injections of varying amounts of scarlet fever toxin. Incomplete general antitoxic immunity hastens the appearance and evolution of the skin reactions to intradermal injections of the skin test toxin. Incomplete local antitoxic immunity causes the site affected to show a more rapid appearance of the rash and earlier blanching than nonimmune portions of the skin during an attack of scarlet fever. This recrudescence of color followed by early blanching in the area of a previously positive skin test for susceptibility to scarlet fever is a specific immune reaction helpful in diagnosis of doubtful rashes. When repeated skin tests for susceptibility to scarlet fever are made on the same person it is advisable to avoid areas of previous injections to prevent modification of the reaction by partial local antitoxic immunity.

FROM AUTHORS' CONCLUSIONS.

EFFECT OF SPLENECTOMY AND BLOCKAGE ON THE PASSIVE TRANSFER OF ANTIBODIES AGAINST TRYPANOSOMA LEWISI. W. H. TALIAFERRO, J. Infect. Dis. **62**:98, 1938.

Bartonella-infected rats were generally used with a view to enhancing the effects of splenectomy and blockade in the experiments reported in this paper. Passive transfer of ablastin (the reproduction-inhibiting antibody) takes place as

effectively in splenectomized rats intensively blockaded with india ink as in normal rats. Passive immunity obtained with ablastin lasts for only a few days. In normal rats it is augmented and replaced by a developing active ablastic immunity, whereas in splenectomized and blockaded rats active ablastic immunity does not develop to any marked extent. In contrast to results with ablastin, the passive transfer of the trypanocidal antibody is slightly but definitely impaired in splenectomized and blockaded animals as compared with normal rats. Passive immunity obtained with the trypanocidal antibody lasts for only a few days. In this case also, the few trypanosomes which may survive do not reproduce in normal rats owing to the development of an active ablastic immunity, whereas they actively multiply in splenectomized and blockaded rats since ablastic immunity fails to develop. Trypanosomes sensitized with specific trypanocidal antibody and injected intravenously are removed from the circulation as quickly in splenectomized and blockaded as in normal rats, but this does not prove that the eventual disposal of the parasites is identical because the parasites may be agglutinated and mechanically removed.

FROM AUTHOR'S SUMMARY.

IMMUNOLOGIC SPECIFICITY OF POLYSACCHARIDE FRACTIONS FROM SOME COMMON PARASITIC HELMINTHS. D. H. CAMPBELL, J. Parasitol. **23**:348, 1937.

Methods are described for the isolation and purification of the antigenic polysaccharide fractions of helminths. Polysaccharides thus prepared from Ascaris lumbricoides, Ascaris suis, Parascaris equorum, Toxocara canis, Maeracanthorhynchus hirudinaceus, Cysticercus fasciolaris and Monezia expansa were tested with antiserums against A. lumbricoides, A. suis, T. canis and M. expansa by the precipitin ring test.

The specificity of this reaction is far greater with polysaccharides than with saline extracts of whole worm material. Whereas saline extracts of whole worm material gave precipitin reactions with heterologous serums, polysaccharide fractions reacted only with homologous antiserums. This specificity was observed even with the closely allied forms A. lumbricoides and A. suis.

FROM AUTHOR'S SUMMARY.

CHEMICAL CONSTITUTION AND BIOLOGIC PROPERTIES OF THE ENDOANTIGEN OF THE BRUCELLA GROUP OF MICRO-ORGANISMS. R. B. PENNELL and I. F. HUDDLESON, Medical Bulletin 156, Michigan State College, Agricultural Experiment Station, 1937.

A method is described for the preparation from cells of Brucella abortus, Brucella melitensis and Brucella suis of highly antigenic fractions, the endoantigens, which are toxic for normal guinea pigs and which precipitate immune serum in dilutions of from 1 : 500,000 to 1 : 5,000,000.

The endoantigens obtained from the three species of Brucella are grossly similar. An endoantigen comprises roughly 25 per cent of the bacterial cell. While containing the same or similar constituents, however, the endoantigens from the three organisms have been shown to differ markedly in the distribution of some of these constituents.

Positive reactions are given to the Molisch test, the biuret test, Millon's test and Bial's test, also a very slight reaction to the Rosenheim test. The nitrogen content of the fraction varies from 6 to 8 per cent. Reducing sugars are absent before hydrolysis. Calculated as dextrose after hydrolysis, reducing sugars represent from 4 to 12 per cent of each endoantigen. Aminonitrogen, phosphorus and sulfur are absent. In the determination of acetyl groups, distillable acid representing an average of 6 per cent of the endoantigen is obtained. This acid is presumably acetic, although that product has not been isolated.

From endoantigen there may be extracted by acetone and ether a compound having the properties of a diketone and an acetone-soluble, saturated liquid fatty acid. These two compounds represent from 10 to 15 per cent of the fraction.

The acetone-ether extracted product still reacts positively to the aforementioned qualitative tests. Tryptophan and tyrosine have been found to represent 18.92 and 8.45 per cent, respectively, of the extracted endoantigen of Brucella melitensis, 11.46 and 0 per cent of that of Brucella abortus and 7 and 9.29 per cent of that of Brucella suis.

From the remaining 65 to 70 per cent of the original fraction there has been obtained an unidentified nitrogenous fraction and an optically inactive sugar acid. These are obtained in quantities such as to preclude the occurrence of any further compounds in significant amounts.

Endoantigen is shown to be relatively stable in the presence of dilute acid and dilute alkali on heating and on long standing. Its activity is not completely destroyed by hydrolysis with dilute acid but is destroyed by similar treatment with dilute alkali. The ability to precipitate specific serum is lessened by extraction with acetone and ether but is enhanced by acetylation or by treatment with 25 per cent NH_4OH. The toxicity and antigenicity of endoantigen are shown to be dependent on proper dosage, an overdosage as well as an underdosage giving poor results. The toxicity and antigenicity are increased by lipid extraction of the endoantigen. Acetylation causes a distinct decline in toxicity but a marked increase in antigenicity.

Endoantigen elicits specific skin reactions in sensitized animals, the lipid extracted and the acetylated endoantigens showing some species specificity in this reaction. Injections of endoantigen have failed to protect normal guinea pigs on subsequent exposure to virulent Brucella organisms and have failed to alter the course of the disease in infected animals. Experiments with cattle and human beings, however, seem to promise immunizing and therapeutic value to endoantigen.

Injection of endoantigen causes hyperglycemia, followed by hypoglycemia, in experimental animals. The basal metabolism is at first stimulated and then depressed. Leukopenia, chiefly due to disappearance of neutrophils from the peripheral blood, follows injection of endoantigen into normal guinea pigs.

Endoantigen may be produced from the previously described albuminoid fraction of Brucella, thus accounting for the toxicity of that fraction and suggesting that this albuminoid is a combination of endoantigen with a protein-like group. Endoantigen is shown to be similar to or possibly identical with the previously described S substance, the latter being probably a partially hydrolyzed endoantigen.

CHORIOALLANTOIC GRAFTS OF LIVER. C. L. OAKLEY, J. Path. & Bact. **46**:109, 1938.

Embryonic chick liver, adult hen liver, embryonic duck liver and fetal rat liver grow in the chorioallantois of the chick. The degree of growth and of reaction to the graft depends almost entirely on the degree of individualization of the transplanted material. The growth consists almost entirely of liver cells; bile ducts are few and usually dissociated from the liver cells, which are trabeculated, show bile capillaries and are separated by sinusoids. No transition from bile ducts to liver cells has been observed. Erythropoietic masses are occasionally present. Both bile ducts and liver cells secrete. Development of liver cells is favored at the expense of development of bile ducts.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL SYMMETRICAL CORTICAL NECROSIS OF THE KIDNEYS PRODUCED BY STAPHYLOCCOCUS TOXIN. S. DE NAVASQUEZ, J. Path. & Bact. **46**:47. 1938.

The priority of the vascular lesion in symmetric cortical necrosis of the kidneys has been established by experiment, confirming previous histologic observations. The vascular lesion consists in acute necrosis of the media of the intralobular and

afferent arteries of the peripheral renal cortex and is unaccompanied by thrombosis. The medial necrosis is preceded by dilatation of the afferent arterioles and glomerular capillaries. There is no evidence that vasoconstriction or spasm occurs. The vasodilatation is the result of loss of muscular control caused by paralysis of the affected arteries and leads to obstruction of the glomerular circulation. The mechanism of the obstruction is thought to be the rapid loss of plasma through the grossly dilated glomerular capillaries due to heightened pressure in the renal artery. The resulting concentration of red corpuscles leads to circulatory stasis with consequent ischemic necrosis of the parenchyma.

FROM AUTHOR'S CONCLUSIONS.

IMMUNIZATION OF CYNOCEPHALIC MONKEYS AGAINST TUBERCULOSIS BY THE SMOOTH VARIETIES OF TUBERCLE BACILLI. L. NEGRE and J. BRETEY, Ann. Inst. Pasteur **59**:173, 1937.

Two monkeys given, respectively, three intravenous injections of 0.01, 0.1 and 1 mg. of smooth tubercle bacilli at monthly intervals showed no lesions after being inoculated with virulent human tubercle bacilli.

A monkey which received a single intravenous injection of 1 mg. of smooth culture showed no macroscopic lesions following an injection of virulent organisms. One given a subcutaneous injection of 10 mg. and later an intravenous injection of 1 mg. showed as a result only four tubercles.

A monkey given ten subcutaneous injections of 0.01 mg. of smooth bacilli showed only four tubercles five months after an injection of virulent bacilli.

Two monkeys which received a single subcutaneous injection of 10 mg. were not protected against virulent organisms, although the lesions were not as pronounced as those in the control animals.

The intravenous route seems superior to the subcutaneous; repeated injections seem superior to a single injection.

FROM AUTHORS' SUMMARY.

DISTRIBUTION OF THE PROPERTIES A_1 AND A_2 IN BELGIUM. P. MOUREAU, Ann. de méd. lég. **17**:873, 1937.

Among 500 persons of blood groups A and AB the following distribution of the subgroups was found: A_1, 374 (74.8 per cent); A_2, 88 (17.6 per cent); A_1B, 25 (5 per cent); A_2B, 13 (2.6 per cent). This distribution of the subgroups is similar to that found in most other populations thus far examined.

A. S. WIENER.

BLOOD GROUPS OF CHIMPANZEES. P. DAHR and R. ROMMEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:494, 1937.

Dahr and Rommel studied the blood cells and serums of 5 chimpanzees. Solutions of agglutinins made according to the method of Landsteiner established that 2 of the chimpanzees had the A and the other 3 the O factor in their blood. The O property was identical with human O and not due merely to absence of A or B. That was established by specific anti-O serums. The serums contained the proper agglutinins; the relation of the agglutinogens and agglutinins complied with the rule of Landsteiner. While human serums frequently contained anti-monkey species agglutinins, the serums of monkeys did not contain antihuman species agglutinins. None of them contained the factors MN. This report brings the number of blood group determinations in anthropoid apes to 111 (89 chimpanzees, 4 gorillas and 18 orang-utans). Of the 89 chimpanzees, 77 belonged to group A and 11 to group O. One could not be classified. Of the 18 orang-utans, 7 belonged to group A, 8 to B and 3 to AB. The 4 gorillas belonged to group A, although, according to Dahr and Rommel, there is some question about the reliability of the determination in 3 of them.

I. DAVIDSOHN.

COMPLEMENT IN NEWBORN CHILDREN, IN INFANTS AND IN FETUSES. P. SÖLLING, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:15, 1937.

The titer of the complement varied little with adults. Very marked deviations in either direction were remarkably rare. Low values appeared more frequent with syphilitic persons. With 20 infants (first year of life) the values were almost the same as with adults and were only slightly higher than those with 93 mature newborn children. With 28 fetuses the values differed according to the age. Complement was detectable from the fourteenth week on but was quite weak until the twenty-eighth week, after which the titers came close to those of mature newborn children.

I. DAVIDSOHN.

ACTION OF EPINEPHRINE AND OF EPHEDRINE ON ANAPHYLACTIC REACTIVITY. F. E. HAAG, H. KOENIG, H. KANN and G. WOLTERS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:22, 1937.

Guinea pigs sensitized with extracts of peas and treated repeatedly with epinephrine showed more severe anaphylactic reactions than controls that did not receive epinephrine. It is concluded that epinephrine increases the anaphylactic reactivity of animals and that after prolonged administration it similarly raises the degree of hypersensitiveness in man. Aqueous and alcoholic extracts of ephedra vulgaris decreased slightly the anaphylactic reactivity of guinea pigs that were actively sensitized with extracts of peas. After extended administration the inhibitory action vanished. Repeated injections of the drug itself failed to sensitize the guinea pigs. Administration of ephedrine shortly or immediately before the shocking injection of the protein did not affect the anaphylactic shock.

I. DAVIDSOHN.

Tumors

EFFECT OF EXTRACTS OF DIFFERENT ORGANS AND TISSUES ON THE VIABILITY OF TRANSPLANTABLE TUMORS. K. SUGIURA, Am. J. Cancer **32**:126, 1938.

An investigation has been made of the effects of immersing fragments of mouse sarcoma 180 and of Flexner Jobling carcinoma in aqueous extracts of fresh rat heart, lung, liver, kidney, spleen, brain, leg muscle, testis, embryo and placenta prior to transplantation. The growth capacity of these fragments was markedly inhibited. The inactivation by extracts of embryo, kidney, heart, liver, leg muscle, brain and testis was less than that by splenic extract. The extract of lung and that of placenta were least toxic but were more toxic than the serums of mouse, rat, guinea pig, rabbit and man. No significant difference was found in the growth-inhibiting action of aqueous extracts of spleens from normal rats and from rats bearing progressively growing Flexner-Jobling rat carcinoma.

FROM AUTHOR'S SUMMARY.

GLIONEUROMA AND SPONGIONEUROBLASTOMA, FORMS OF PRIMARY NEUROECTODERMAL TUMORS OF THE BRAIN. J. H. GLOBUS, Am. J. Cancer **32**:163, 1938.

A relatively large group of primary tumors of the brain, neuroectodermal in origin and supratentorial in location, which have hitherto been regarded as rare, are described under the designations "glioneuroma" and "spongioneuroblastoma." The glioneuromas are anatomically relatively benign and may lend themselves to successful surgical intervention, while the spongioneuroblastomas are more malignant, and their timely recognition may spare the patient and the surgeon alike unnecessary and hazardous surgical intervention. A survey of the clinical histories in cases of each form of tumor yields some diagnostic leads for the recognition of the anatomic character and the precise localization of each of these forms. The proper evaluation of the histologic character of each of these forms of tumor gives promise of clarifying the understanding of the genesis of primary tumors of the brain. The presence of both spongioblastic and neuroblastic derivatives in

these tumors lends support to the Cohnheim embryonal rest theory of tumor formation. The frequent finding of the heterotopic and histiotopic lesions of tuberous sclerosis in association with the more malignant form, the so-called spongioneuroblastoma, and the equally frequent finding of other cellular patterns similar to those occurring in the infant's brain are other important features favoring this theory.

FROM AUTHOR'S CONCLUSIONS.

BIOELECTRIC PROPERTIES OF CANCER-RESISTANT AND CANCER-SUSCEPTIBLE MICE. H. S. BURR, G. M. SMITH and L. C. STRONG, Am. J. Cancer **32**:240, 1938.

In this study is presented evidence of (1) bioelectric differences between cancer-susceptible and cancer-immune mice; (2) a correlation of the electrical pattern with the age of the mice; (3) a characteristic change in the electrodynamic field of a mouse incident to the development of cancer; (3) a gradient in potential between the tumor and the normal areas of the organism, notably during the early development of the tumor.

FROM AUTHORS' SUMMARY.

ON THE PROLIFERATIVE CHANGES TAKING PLACE IN THE EPITHELIUM OF THE VAGINA AND CERVIX OF MICE WITH ADVANCING AGE AND UNDER THE INFLUENCE OF EXPERIMENTALLY ADMINISTERED ESTROGENIC HORMONES. V. SUNTZEFF, E. L. BURNS, M. MOSKOP and L. LOEB, Am. J. Cancer **32**:256, 1938.

The changes which take place in the vagina and cervix with advancing age and under the influence of long-continued experimental stimulations by estrogenic substances, which ultimately may lead to the production of carcinoma-like proliferations, are not compatible with the view that somatic mutations in the stimulated tissues are the immediate cause of their cancerous transfusion.

FROM AUTHORS' SUMMARY.

CHRONIC RADIODERMATITIS. T. S. SAUNDERS and H. MONTGOMERY, J. A. M. A. **110**:23, 1938.

A clinical study was made of 259 cases of chronic radium or roentgen injuries to the skin. The large majority of injuries seen today are contracted through therapeutic exposure rather than occupationally, as in former years, chronic radiodermatitis being encountered particularly as a result of the injudicious irradiation of various benign dermatoses. Carcinoma developed in 21 of the cases, and the principle that "the more extensive the injury, the more likely the development of cancer thereon" is well established. All the carcinomas were of the squamous cell type, and the majority showed the phenomena of individual cell keratinization and of having begun as a carcinoma in situ. Carcinoma apparently develops with equal frequency from either keratoses or ulcerations. The prognosis depends on the degree of malignancy and one complete and radical removal.

Chronic radiodermatitis has a definite and characteristic histologic picture. Keratoses should be thoroughly destroyed by electrosurgical measures, and ulcers should be excised and the procedure followed by skin grafting. Amputation of a digit or extremity is definitely indicated whenever malignant degeneration has occurred and whenever the lesion cannot be removed completely by wide excision. The early recognition and prompt treatment of "precancerous" radiation dermatoses (and ulcers) would go a long way toward preventing the subsequent development of carcinoma.

FROM AUTHORS' SUMMARY.

PRODUCTION OF THE SHWARTZMAN PHENOMENON BY MEANS OF BACTERIAL AND TUMOR EXTRACTS. W. ANTOPOL, J. Infect. Dis. **61**:331 and 334, 1937.

Extracts of Bacillus typhosus, Bacillus coli, Pneumococcus type III, meningococcus, Bacillus dysenteriae (Flexner and Sonne) and Bacillus proteus (vulgaris and ×19) were capable of producing the Shwartzman phenomenon. These

extracts could be used interchangeably with one another and with filtrates of B. typhosus, i. e., an extract from one source for the skin preparatory dose and an extract from another source for the provocative dose. A provocative dose of 0.0005 mg. of the extract of B. proteus could produce a severe reaction after preparation with 1 mg. or more of the same extract. With a larger intravenous dose, a smaller intradermal dose was capable of preparing the skin site.

Skin preparatory factors may be present in tumor tissue. The existence of such factors may explain the strong hemorrhagic reactions which can be induced in tumors by an intravenous injection of a provocative dose without any preliminary preparation. Provocative factors may also be present in tumor tissue.

From Author's Summaries.

Influence of Transmitted Leukemia. J. Victor and J. S. Potter, Brit. J. Exper. Path. **19**:227, 1938.

The intraperitoneal inoculation of a saline suspension of leukemic spleen or lymph nodes of two different transmission lines depressed the anaerobic glycolytic rates but not the respiratory or aerobic glycolytic rates of uninfiltrated lymphoid tissue. The inoculation of similar amounts of normal spleens of two different strains of mice produced no such effect. No inhibition of anaerobic glycolysis could be demonstrated when normal and leukemic lymphoid cells were mixed in Ringer solution, or when normal cells were suspended in Ringer solution that had previously bathed leukemic cells. The inhibition of the anaerobic glycolytic activity of uninfiltrated lymphoid tissue of mice with transmitted lymphatic leukemia depends on the following factors: (1) a specific response to individual transmission lines, (2) the interval after inoculation and/or the quantity of leukemic tissue in the host and (3) a humoral factor which is either a product of leukemic cells or the resultant of a reaction between host and leukemic cells.

From Authors' Summary.

The Transmission of the Rous Filterable Agent to the Normal Tissues of Fowls. E. Mellanby, J. Path. & Bact. **47**:47, 1938.

When the fowl has a Rous sarcoma, the Rous agent is widely diffused throughout the body. The presence of this factor can often be demonstrated in the spleen, liver, muscle, brain and other organs by the production of Rous sarcoma when the cells or cell-free filtrates of these organs are injected into other fowls. It is unusual to find evidence of Rous virus in the organs and tissues of a Rous tumor–bearing fowl until the seventh day after the injection of the Rous cells, i. e., until after the new growth is established. It is easiest to demonstrate the presence of Rous virus in the organs of a Rous tumor–bearing fowl between the tenth and the eighteenth day after injection. As a rule, it is again more difficult to demonstrate the presence of the virus of the Rous tumor in liver, spleen and other tissues later than the twentieth day, i. e., when the fowl may be approaching death, even when metastases are present. A method of antagonizing the Rous agent often seems to have developed by this time. The spleen and liver of the tumor-bearing fowls can be dried and ground and still display tumor-inducing properties on injection, resembling in this respect the Rous sarcoma itself. After washing out the blood of the sarcomatous fowls, or of fowls which have received an intravenous injection of the Rous agent, with Ringer's solution, the injection of cells of the spleen and liver may still induce growths in other birds, indicating that the Rous agent enters the cells of these organs. After one intravenous injection of a cell-free filtrate of Rous sarcoma has been given to fowls, the presence of the Rous virus can be demonstrated in the spleen at all periods up to five days, suggesting that the virus injected is not reduced in amount or in effectiveness in this time. It is also present in the spleen after five days, but the usual initiation of a new growth at the site of injection about this time prevents further deductions being made as to the fate

of the initially injected Rous agent. In 2 resistant fowls no Rous agent could be found in the tissues sixteen and forty-one days, respectively, after intravenous injection. FROM AUTHOR'S SUMMARY.

THE OCCURRENCE OF ONCOCYTES IN VARIOUS NORMAL ORGANS AND IN TUMORS OF THESE ORGANS. H. HAMPERL, Virchows Arch. f. path. Anat. **298**:327, 1936.

In previous studies Hamperl described in normal salivary glands and in tumors of salivary glands a type of cell to which he gave the name oncocyte. It is an epithelial cell characterized by a size larger than that of the neighboring normal cells, with a cytoplasm filled with acidophilic granules. The nucleus is pyknotic, sometimes angular, apparently senile and situated away from the base of the cell. In the present detailed communication the previous findings relating to the salivary glands are reviewed, and the presence of oncocytes is described in the normal pancreas, parathyroids (oxyphilic cells), hypophysis, thyroid and fallopian tube. Somewhat similar cells, of whose nature the author is not certain, have been noted in the liver and testis. The oncocytes result from alteration of normal, differentiated epithelial cells not by a process of dedifferentiation or involution, as some have maintained, but by direct transformation. They are not present in childhood and occur in increasing numbers with advancing age. Hamperl believes that aging of the organs is the most important factor in the occurrence of oncocytes. He then describes similar cells, in variable numbers, in tumors, especially benign ones, of the organs in which oncocytes normally occur. While multiplication of preexisting oncocytes is admitted as a possibility to explain the presence of the cells in tumors, Hamperl accepts as offering a more likely explanation the possibility of an abnormal and more rapid process of aging of differentiated cells under the influence of the abnormal conditions that exist in tumors. The word "oncocyte," according to its derivation, means tumor cell. In his earlier publications on the salivary glands the author left the impression that the oncocytes give rise to mixed tumors of the salivary glands, in which event the term "oncocyte" would be a proper one. His present standpoint seems to be different. Oncocytes occur in tumors but apparently do not give origin to tumors. Hamperl objects to the term "oncocytoma," which some American writers have used.

O. T. SCHULTZ.

THE PLACE OF RETOTHEL SARCOMA IN THE TUMORS OF THE LYMPH NODES. G. DE OLIVEIRA, Virchows Arch. f. path. Anat. **298**:464, 1936.

A histologic study of 20 tumors of reticuloendothelial origin involving lymph nodes forms the basis of this 51 page contribution. In the embryonic development of the reticulum of the parenchyma of lymph nodes four phases or stages are described: (1) an afibrillar cytoplasmic syncytial stage in which the nuclei are embedded, cell outlines are not evident, and argentophilic or other fibrils are not present; (2) a fibrillar cytoplasmic syncytial stage, in which separation of individual cells begins, the cells being united, however, by short blunt processes, and in which the formation of intracellular argentophile fibrils is evident; (3) a reticulosyncytial stage, in which some syncytium is still present though most of it has been largely transformed into distinct cells, with a network of intercellular reticulum fibrils, and (4) a final stage of fibrocellular differentiation, with a rich reticulum, to which the cells are applied, and with collagen fibrils. The author's 20 tumors are divided into six groups according to the degree of differentiation shown by the tumor tissue. The first four groups closely simulate in type the four stages of development of lymphadenoid reticuloendothelium and have received the same names as applied to the developmental stages. The fifth group consists of tumors with a greater degree of variation in the size and shape of the cells than is seen in normal lymphoid tissue; this is termed the polymorphocellular type of retothel sarcoma. The fourth group consists of tumors in which the

reticuloendothelium attains its highest differentiation into hemopoietic tissue; in 3 of the 4 tumors of this group there was differentiation into lymphocytes, and in the fourth, into plasma cells. This group establishes a relationship between the retothel sarcomas of lymph nodes and reticuloendothelial tumors of bone marrow. Because of their tendency toward generalization, the prognosis of retothel sarcomas is bad. Most of these tumors arise in the upper half of the body, but they may originate in the abdominal cavity. The latter are more malignant than those arising above the diaphragm.

O. T. SCHULTZ.

RELATIONS BETWEEN NUCLEAR SIZE AND TUMOR GROWTH. E. SCHAIRER, Ztschr. f. Krebsforsch. **45**:279, 1937.

In a series of observations of nuclear dimensions in the skin of normal and of tarred mice and in tar-induced papillomas and carcinomas, Schairer failed to find evidence of the Ehrich phenomenon of doubled or quadrupled volume with onset of malignancy. He concludes that the observation is not universally applicable and that there should be grave reservations in the practical and theoretic conclusions that may be drawn from it. Results that in general were similar were obtained in the measurement of liver cells in tarred mice, with the thought that increases, if present, would possibly be evidence of the onset of cancerous predisposition.

H. E. EGGERS.

SPLENIC METASTASES AND LYMPHOGRANULOMAS OF THE SPLEEN. B. SCHMID, Ztschr. f. Krebsforsch. **45**:298, 1937.

In a discussion of the rarity of splenic metastases of malignant tumors, Schmid points out that other organs are also rarely involved. But with most of these organs this can be explained by circulatory peculiarities, whereas such obstacles do not operate in the spleen, where infarction is especially common. He describes in some detail 2 cases of splenic involvement. In a case of double carcinoma of the colon and stomach, the spleen was invaded both by extension and by transmission of tumor tissue in the lymph or blood. The latter lesions were encapsulated with connective tissue, through which the tumor was penetrating into the adjoining splenic tissue. In the second case, a gross anatomic diagnosis was made of carcinoma of the ascending colon. The tumor, however, proved to be a lymphogranuloma with metastatic involvement of the regional lymph glands and of the spleen. Still another case is cited in which an indeterminate picture in the spleen, regarded as that of lymphogranuloma, was associated with the occurrence elsewhere of generalized carcinomatosis of gastric origin.

H. E. EGGERS.

EFFECT OF GROWTH-PROMOTING AND OF GONADOTROPIC HORMONES ON LEUKEMIA AND SARCOMA IN FOWLS. J. ENGELBRETH-HOLM, A. R. MEYER and E. UHL, Acta path. et microbiol. Scandinav. **14**:481, 1937.

The growth-promoting factor proved incapable of accelerating the development of either hemocytoblastosis or sarcoma in fowls. An acceleration was hardly to be expected, according to the authors, as both the strains examined were extremely virulent. On the other hand, when tumors were produced by injections of 1,2,5,6-dibenzanthracene a higher percentage of fowls treated with the growth-promoting factor than of the untreated fowls responded with "takes." This result, however, is a little doubtful as the number of fowls used in the experiment was small. The gonadotropic substance was unable to inhibit the development of hemocytoblastosis, whereas it did inhibit the growth of transplanted sarcoma to some degree, the tumors in the treated animals attaining only to half the size of the tumors in the control groups.

FROM AUTHORS' SUMMARY.

CANCER OF THE LUNG. F. HARBITZ, Norsk mag. f. lægevidensk. **98**:1451, 1937.

This article gives the results of a study, mainly morphologic, of 29 cases. Harbitz is inclined to doubt whether conclusive evidence of a real increase in the incidence of cancer of the lung in recent times has been produced. He holds that the apparent increase may be explained by improvement in clinical and anatomic diagnosis and by the increase in the average length of life. In the years 1919 to 1934 in Norway 353 cases of cancer of the lung were reported, 183 in men and 170 in women. The Norwegian experience does not indicate that cancer of the lung is much more frequent in men than in women. In Norway pulmonary cancer occurs all over the country, in rural districts as well as in cities, and without any obvious predilections or special occupational relations. The article is accompanied by a good English summary.

Medicolegal Pathology

HEREDITY OF THE SUBGROUPS OF GROUPS A AND AB. P. MOUREAU, Ann. de méd. lég. **17**:875, 1937.

The inheritance of the subgroups was studied in 30 families with 84 children. Not a single exception to the theory of Thomsen, Friedenreich and Worsaae was encountered.

A. S. WIENER.

DIFFERENTIATION OF VITAL AND POSTMORTEM CHANGES IN DEATH DUE TO HANGING. H. BLUM, Virchows Arch. f. path. Anat. **299**:754, 1937.

This work, done at the medicolegal institute of the university in Halle, Germany, is based on 15 cases of suicide by hanging. It attempts to differentiate between changes brought about during life by the act of hanging and those occurring in a body hung after death to simulate suicide. The subject had been previously discussed in Hungarian by Orsós, whose work the present author evidently follows closely. Alterations of the skin of the strangulation furrow, such as partial shredding of the horny layer and compression of the cellular layer of the epidermis, formation of vesicles, capillary extravasations, compression of the vessels of the skin in the furrow and dilatation above, all may be observed in a dead body hung before rigor has set in. In the subcutaneous connective tissue Orsós described metachromatic staining with the Mallory stain. Blum noted similar metachromatism with the Petersen acid–alizarin blue stain but claims that it is not necessarily a vital change. In what Blum calls the internal strangulation furrow, seen in the muscles of the neck, swelling of striped muscle fibers, with loss of cross striation and waxy change, occur, but such changes too are not always vital phenomena. As a characteristic change Orsós described emulsification of fat beneath the strangulation furrow. The single large fat globule of the cell is emulsified into many minute droplets, which do not fuse again because each is surrounded by a film of protein derived from the plasma expressed from the capillaries. The change is a physical one brought about in the living fat cell by the force of the strangling agent. Such emulsification or homogenization of fat is, according to Blum, the only alteration that cannot be duplicated in a body hung after death. It is not seen in every death due to hanging and therefore has differential value only when present.

O. T. SCHULTZ.

CARDIAC RIGOR MORTIS. P. SCHNEIDER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:168, 1938.

The experiences of several pathologists in the World War have led to statements that rigor mortis in the heart lasts from twelve to twenty-four hours. Haberda claimed that the duration is between fourteen and fifteen hours for the left side of the heart. Meixner, however, expressed the opinion that the total period of rigor was more nearly six and one-half hours, although he had encountered hearts in which the rigor lasted from two to three days. Various factors influence the onset and duration of rigor. In general, rigor sets in more quickly

and remains longer after an accident or a short illness. Following sudden death, rigor is more intense than when death occurs gradually, owing to the fact that in sudden death the heart muscle is stopped in action in full vigor. After a mechanical injury of the heart, such as a gunshot wound or a rupture, an intense and long rigor is noted, especially if the heart involved by such a circumscribed lesion is a powerful normal one. The absence of rigor is noted in only a few cases, as extensive cardiac injury, multiple infarctions, marked fatty degeneration, some septic conditions and certain chronic metal poisonings. It is obvious, therefore, that rigor in the heart can be used along with other important postmortem evidence to determine the time of death. Caution, however, must be employed in using as the only index a criterion so frequently influenced by other factors.

GEORGE J. RUKSTINAT.

EXPERIMENTAL OBSERVATIONS ON MOTORCYCLISTS WITH ALCOHOLIC INTOXICATION. H. BAUER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:193, 1938.

Alcohol in the blood in concentrations of from 0.7 to 0.9 mg. per hundred cubic centimeters affects the driving of motorcyclists. As has been stated previously, the finer cerebral functions are altered before definite clinical symptoms of intoxication appear. In the operation of a motorcycle, two things are important: what the rider believes in his own mind and what he actually sees. Since small doses of alcohol alter peripheral vision in far greater degree than central vision, warning signals, road crossing signs and like objects will be overlooked. Small doses of alcohol also affect binocular vision so that the subconscious mind has difficulty in evaluating size and distance. Kraepelin and his students have shown that alcohol in concentrations of no more than 1 per cent in the blood produce disturbances in attention and a prolongation of reaction time.

Bauer devised four types of experiments to test the influence of alcohol on motorcycle riders. All the tests were performed first by eight sober riders. These riders then imbibed enough alcohol to produce concentrations not higher than 0.9 mg. per hundred cubic centimeters of the blood and were again submitted to the tests.

The first test consisted of riding along a straight line 30 meters long and 30 cm. wide. After drinking, the riders frequently rode off the line.

The second test was a test of attention. The cyclists were asked to note two vehicle license numbers in four places near an oval track.

In the third test, execution of two figure 8's, 8 meters across, and looping through arches 1 meter wide were investigated. The figure 8's formed after the senses were benumbed slightly by alcohol were in some instances jerky, and the standards outlining the arches of contiguous loops were struck in several instances.

The fourth test was performed by riding along a path on which a switch was operated by the front wheel of the motorcycle. This switch caused a light to flash either left or right, and the distance traversed after receiving this signal was the index of the reaction period of the rider.

A questionnaire filled in by each participant indicated that small doses of alcohol gave riders a sense of security so that they rode faster.

GEORGE J. RUKSTINAT.

ACUTE POISONING BY THORIUM X. H. HAMPERL, Virchows Arch. f. path. Anat. **298**:376, 1936.

A woman aged 26 years with suicidal intent took a solution containing 40,000 electrostatic units of thorium X. Both the erythrocytes and the leukocyte count rose during the first twenty-four hours, the latter to 22,300. The erythrocyte count then dropped and maintained an approximately normal level throughout the rest of the illness. The leukocytes decreased in number progressively and rapidly and practically disappeared from the peripheral blood by the eighth day. This low level was maintained in spite of repeated blood transfusions, throughout the rest of the

illness. Death occurred on the sixteenth day. Autopsy revealed a diffuse pseudomembranous enterocolitis. In the bone marrow the chief effect was on the granulocytes. The chromosomes of the dividing cells were swollen and fused with each other, resulting in death of many cells and atypical division of others. The injured cells resulting from such divisions were short lived. The author could find record of only a single previous case of fatal acute irradiation intoxication. He reports also a case of chronic damage from irradiation in a 35 year old roentgenologist who had not properly protected himself. Eight months before death it was discovered that he had severe anemia of the aplastic type. One month before death the granulocytes disappeared from the peripheral blood. Terminal manifestations were pneumonia and ulcerative tonsillitis. The bone marrow was fibrotic. Previously reported cases of fatal chronic damage from irradiation are summarized.

O. T. SCHULTZ.

Society Transactions

NEW ENGLAND PATHOLOGICAL SOCIETY

FREDERIC PARKER JR., *President*

May 25, 1938

J. B. HAZARD, *Secretary*

BENIGN ENLARGEMENT OF THE PROSTATE. ROBERT A. MOORE (by invitaiton), Cornell University Medical College, New York.

The disease of the prostate which produces urinary obstruction in elderly men has been known since the earliest days of medicine and has been designated by a number of names. The term "benign enlargement" seems best, since the word "enlargement" is noncommittal as to the nature of the process, which is at present unknown.

A statistical study of the material I have encountered and of published observations reveals the following pertinent facts concerning the incidence of benign enlargement: It is a disease of the presenile period of life; the greatest incidence is in the seventh decade; there is a progressive increase in incidence with increase in age; it occurs with a significantly greater frequency in married men.

The smallest, and presumably the earliest, lesions are found (1) in the periurethral tissue, (2) about the prostatic ducts and (3) in the lateral and middle lobes of the prostate. In the first two locations the essential alteration is a nodular proliferation of muscle tissue and fibrous tissue. In the third there is simultaneous proliferation of epithelium and stroma. As the nodules increase in size, surrounding glands are compressed, and in many instances the epithelium on the inner side of the gland undergoes hyperplasia. These encircling slitlike glands with proliferation of the epithelium next to the nodule constitute the most characteristic histologic feature of benign enlargement. The developed nodules are free from elastic fibrillae, which are abundant in the normal prostatic stroma. Both inside and outside the nodules are numerous small foci of lymphoid tissue. These foci do not represent chronic inflammation, because the reticulum within them is characteristic of lymphoid tissue and not of chronic inflammatory tissue. Nodules are formed only about and in glands whose ducts empty into the urethra on a level with or above the verumontanum.

There is histologic evidence suggesting an irregular stimulation of the prostate during presenility, namely: a variation in the type of epithelium, atrophy of the epithelium, the presence of tall columnar epithelial cells and fibrosis of the stroma.

In other glands, notably the breast, atypical hyperplasia and nodule formation have been associated with malignant change. In the prostate I find no evidence for this relationship. I have encountered only 2 cases in which there was indisputable evidence that a carcinoma started in an area of benign enlargement. The finding of the two diseases in the same prostate does not prove a causal relationship, since the two processes may have started independently.

In the prostate of the newborn infant there are metaplastic changes in the same ducts which are involved later in life by benign enlargement. In the female pseudohermaphrodite the prostate is represented by a mass of tissue entirely above the verumontanum. This evidence compels one to turn one's attention to the theory that benign enlargement is related to the female phase of sex and to the relative amounts of active "sex hormones" in the body.

Castration brings about changes in the prostate which differ only in degree from those seen during presenility and senility. The studies on the secretion of "sex hormones" in the urine show that there is a gradual decrease in the excre-

tion of androgen and that during all periods of life there is great variation in the amount of urinary androgen from day to day. To study the tissues more carefully my associates and I have employed ocular transplants of the prostate and seminal vesicles. With this method it can be shown that the male secondary sexual organs increase in size markedly after injections of estrogen. The change in size results from congestion, edema and hyperplasia of the stroma. There are numerous mitotic figures in the muscle cells. Estrogen also induces metaplastic changes in the urethra and prostatic ducts of animals.

The prostatic ducts in cases of benign enlargement in men show metaplasia, and the metaplasia is extremely conspicuous in patients given injections of an estrogenic substance.

Though all of the morphologic and experimental evidence presented supports the idea that the estrogenic hormones are concerned in the genesis of benign enlargement, there are some observations which are contrary to this theory. Since the prostatic utricle and the appendix testis are derived from the müllerian duct, the epithelial lining of these two structures should, with effective estrogenic stimulation in patients with benign enlargement, show squamous cell metaplasia, and it does not. That the tissue is capable of squamous cell metaplasia was shown in a case of metaplasia of the utricle which I observed and by Burrows' observation of metaplasia of the appendix testis in a patient with carcinoma of the male breast. In the case which I observed, the autopsy showed complete destruction of both testes and of the pituitary by a carcinoma metastasizing from the stomach.

An explanation of the absence of changes in the utricle and appendix testis is to be found in the neutralizing or blocking action of androgen and estrogen for each other. Thus, an ocular transplant in a castrated rabbit will respond to 30 units of estrogen, while 100 units is required to secure a similar response in an intact rabbit. It is possible that in the presenile man with a decreased quantity of androgen the estrogen acts on the prostate, which is thus assumed to be more sensitive to stimulation than the vestigial utricle and appendix testis.

The final proof that benign enlargement of the prostate is a hormonal disease related to the testis is given by the fact that not a single person with the disease is found among those referred to in 28 published reports on eunuchs and eunuchoids who lived to be more than 45 years of age.

In conclusion, although many problems must await further investigation, I wish to submit this tentative theory as the basis for future study: Benign enlargement of the prostate is a disease of presenility due to the altered and irregular differential secretion of androgen and estrogen during this period of life.

DISCUSSION

FLETCHER COLBY (by invitation), Boston: Dr. Hugh Young in an early paper brought out the infrequency of enlargement of the prostate in single men as indicated in a series of 300 cases of enlargement of this gland.

The prostate and the seminal vesicles enlarge when secretion of the hypophysis is increased. A group of workers in Cleveland have shown that when a castrated animal is joined with a second noncastrated animal through union of the peritoneal cavities, the prostate of the normal animal will show hypertrophic changes. This has been interpreted as due to the increased hypophysial secretion elaborated by the castrated animal, acting through stimulation of the testes of the normal animal.

After "hormonal" treatment, it is said, a little over 50 per cent of the patients showed a recession of symptoms, but there was no decrease in the size of the prostate.

SHIELDS WARREN, Boston: I should like to ask Dr. Moore what happens to the transplants of prostatic and seminal vesicle in the eye. Marked enlargement was shown, together with increased vascularity, but was this enlargement due to the increased blood supply or was there also hyperplasia of the glandular elements and of the stroma? The suddenly changing size of the transplants suggests the possibility that the enlargement was due to a vascular change. It has been my experience that the prostate showing benign enlargement may show a

relative lack of vascularity. After surgical removal of the prostate, infrequently but not rarely one sees an increase in the size of the remaining prostatic tissue. Is there anything in Dr. Moore's experiments that will explain this? Gynecomastia is associated with an enlarged prostate, and it seems that this aids somewhat in proving Dr. Moore's theory.

H. E. MacMahon, Boston: I should like to ask first, whether antuitrin S (a preparation of the gonadotropic substance from the urine of pregnant women) produces enlargement. Second, I should like to know what Dr. Moore thinks of the prostatic infarct with a surrounding zone of squamous cell metaplasia.

A. Reynolds Crane, Boston: I have been wondering what is the relative frequency of infarcts in relation to malignant change. Also what is the relative significance of squamous cell metaplasia? Has there been any attempt to correlate vitamin A deficiency with the squamous cell metaplasia seen in the prostate?

Donald A. Nickerson, Boston: Have assays of the prostate in benign enlargement been done for estrogen content?

Charles Branch, Boston: It has been my general experience that the prostate in benign hypertrophy has been highly vascular. The transurethral method is favored at the Massachusetts Memorial Hospitals. We have found on autopsy that after removal of prostatic tissue lateral lobes replace themselves but the posterior lobe does not enlarge. Instead it leaves a deep depression, which occasionally gives considerable trouble.

E. R. Mintz (by invitation), Boston: I have found in a series of 13 or 14 patients castrated for carcinoma of the penis that there is no evident prostatic enlargement. Dr. Moore's remark in regard to lymphoid tissue in the prostate is interesting. Squamous cell metaplasia may be related to carcinoma in that squamous cell carcinoma has occurred in the prostate. This is in general, however, a rare disease.

Frederic Parker Jr., Boston: I should like to ask Dr. Moore what was the source of the estrogen in the patient in whom both the pituitary gland and the testes had been replaced by carcinoma. Also, I should like to remark on the apparent resemblance of some of the photomicrographs showing changes in glands in the prostate to what has been seen in the endometrium in response to estrogen in one instance and in response to progestin in the other. Have these substances been studied in the urine?

Robert A. Moore (by invitation), New York: Castration apparently has no effect on the tissues involved in benign enlargement. There are a number of cases reported in which a histologic study was made months or years after castration. On the basis of these observations, benign enlargement may be regarded as a tumor because the responses to the normal stimuli for growth and atrophy are lost, and the tissue is autonomous. However, I am not certain that this reasoning is sound.

From morphologic studies, no relation between prostatic enlargement and the pituitary can be established. In 20 cases of benign enlargement which my co-workers and I have studied, the pituitary gland weighed only 20 mg. less than the normal for this period of life.

The results of treatment with the supposed water-soluble factor of the testis, inhibin, are not conclusive. I have studied sections from the prostates of 3 patients treated with inhibin and have found no differences from the usual picture of benign enlargement. Clinically, 50 per cent of patients treated with inhibin are said to be improved. Any evaluation of clinical improvement in benign enlargement must take into account the natural remissions and exacerbations of the disease. Clark followed 100 patients with benign enlargement who refused operation, and they did remarkably well.

The "hormonal" treatment of benign enlargement is reduced to absurdity when reports state that 70 per cent of patients improved after treatment either with an estrogen or with an androgen. The theories on which the use of these two substances are based are diametrically opposed, and if an androgen improves the patients, an estrogen should make them worse.

I agree that no lesion has ever been produced in an experimental animal which is analogous to, or even similar to, benign enlargement in man.

In answer to Dr. Warren: Sections of the ocular transplants show edema and congestion. There is, in addition, hyperplasia of the smooth muscle cells. In man, the injection of up to 1,000 mg. of testosterone propionate or 125,000 units of estradiol benzoate does not bring about any demonstrable edema or congestion of the prostate.

Recurrence of prostatic enlargement should not be considered remarkable. Present day operations are only palliative procedures, and prostatic tissue remains in the body. If the patients live long enough, there is no reason why new nodules should not be formed. There are about 50 well authenticated cases of recurrence in the literature.

Gynecomastia is not significantly associated with benign enlargement of the prostate. There are in the literature reports of 3 cases of the appearance of gynecomastia after prostatectomy. The authors of these reports contend that the prostate secreted a hormone which inhibited the growth of the breast. I find this difficult to believe. A report of a case of chorionepithelioma of the testis and gynecomastia was published in the *Acta pathologica*. The authors stated that the prostate showed hypertrophy, but the photomicrograph shows only the normal prostate of a young man.

In answer to Dr. MacMahon: Infarction and squamous cell metaplasia are also associated with fibrosis of the stroma. I believe the infarction is the result of autochthonous thrombosis. The squamous cell metaplasia has no relation to a malignant tumor. The squamous cell metaplasia of vitamin A deficiency is different and may be seen in the prostates of patients with carcinoma of the esophagus.

The relation between benign enlargement of the prostate and sexual drive is difficult to evaluate. The married state does not signify sexual drive any more than the single state signifies lack of it.

We have no observations on the hormonal content of the prostatic tissue. Deming reported no detectable estrogen, but his methods were not sensitive to small amounts.

In answer to Dr. Branch: The relative merits of the various types of operation have been discussed by urologists since 1900. I feel that the transurethral operation is not a sound surgical procedure; no attempt is made to follow anatomic lines of cleavage. For that operation, except in the hands of a few, the operative mortality is as high as for conservative prostatectomy, and the amount of tissue removed is small. The method of removal, with a burning current, also makes it impossible to carry out satisfactory histologic examinations.

I believe that the perineal prostatectomy offers as much as the suprapubic operation in the treatment of benign enlargement and, in addition, affords an opportunity to treat carcinoma of the prostate. I can add nothing to what Dr. Hugh Young has said and published on this subject. Carcinoma of the prostate is a common disease, and urologists should make some attempt to cure it by radical surgical excision.

Castration invariably results in atrophy of the prostate. The best proof of this statement is found in the observations of Tandler and Gross on castrated persons.

Lymphoid tissue is found in the prostate, and small round cell sarcoma has been reported. Squamous cell carcinoma of the prostate is rare. I have seen it only twice. It is possible that such a tumor is a tumor of the prostatic utricle and not of the prostate. It does not metastasize.

As to Dr. Parker's questions, I can reply only that the source of estrogen in the patient in whom the testis and pituitary had been destroyed by tumor is not known and I can offer no explanation. It is possible that the adrenal may have played a part in the production of the supposed estrogen. Cystic dilatation of the glands is common in the lateral lobe of the prostate in the sixth decade. It is not consistently associated with benign enlargement. It is a definite clinical entity, which prostatic massage and the application of silver nitrate or other substance to the verumontanum may cure. I do not know of any relationship to progestin.

Book Reviews

Atlas of Hematology. By Edwin E. Osgood, M.A., M.D., Assistant Professor of Medicine and Head of the Department of Experimental Medicine, University of Oregon Medical School, Portland, Ore., and Clarice M. Ashworth, Medical Illustrator, University of Oregon Medical School, Portland, Ore. Cloth. Price $10. Pp. 255, with 326 illustrations in color. San Francisco: J. W. Stacey, 1937.

This atlas contains colored figures of 271 immature and mature blood cells, 37 cells with blood parasites (of which 34 are malarial), 8 artefacts and 9 cells of the blood in common conditions. Most of the illustrations are not as sharp and clear as the classic lithographed figures in Pappenheim's atlases. The granules and the nuclei are frequently poorly drawn. The concise text is offered as a help in reaching a rapid differential diagnosis.

Several serious criticisms may be made of the book: 1. It does not live up to some of the claims made for it by the authors. 2. It proposes an extensive new terminology. 3. In the discussion of the origin of the blood cells, it contains mistakes which indicate ignorance of important aspects of the background of the subject.

The reviewer, conscious of the possibility that he is overconservative, prefers to review the book mainly by quotations. On page 9 it is stated that "the following system has been developed to permit anyone to identify any cell encountered in a properly stained preparation." But the authors frequently cannot give absolute diagnoses of the cells that they have pictured, for they say on page 16 that the lymphoblast "can be differentiated with certainty from the granuloblast (myeloblast) (52-58), monoblast (31-33), plasmablast (118), or karyoblast (megaloblast) (132-137) only by the cells associated with it." Obviously, it requires little ability or experience to call a particular stem cell a myeloblast if the blood picture is that characteristic of myeloid leukemia, with great numbers of myelocytes, etc. After reading on page 16 that the lymphoblasts "are not distinguishable in the normal marrow from the more numerous granuloblasts (myeloblasts)," one wonders why these cells are called lymphoblasts and not myeloblasts.

Osgood, the senior author, stands almost alone in denying that there are two series of red blood cells, the primitive cells of the early embryo and the permanent generation of oxygen carriers.

He has introduced a new terminology. This is unfortunate, as hematology is already overly rich in terms. In the reviewer's opinon it would have been better to adopt one of the more or less standard terminologies, such as that of Ferrata or that of Naegeli or that of Downey. The prediction may be ventured that the new terms, "progranulocytes S and A" for promyelocytes, "granulocyte" for myelocyte, "rabdocyte and lobocyte" for polymorphonuclear leukocyte, "plasmablast and proplasmacyte" for the precursors of the plasma cell, "karyoblast, prokaryocyte, karyocyte and metakaryocyte" for the nucleated immature erythrocytes and "akaryocyte" for the erythrocyte, will not be widely used. While adhering strictly to the new terminology in dealing with the leukocytes, Osgood uses the term "erythrocytes" almost exclusively when speaking of the akaryocytes in the section on the anemias.

Each of the following form quotations, from pages 12 to 13, is incorrect and shows a misunderstanding of the points of view of other hematologists, as will be immediately apparent to those versed in hematology: With the frontispiece as a reference for Osgood's views on the histogenesis of the blood cells, one reads, "Note that the first cells of each series are almost identical in appearance. The

monophyletists or unitarians, including Downey, Ferrata, and Pappenheim, believe that these cells are actually identical and use the terms *myeloblast* (Downey), *hemocytoblast* (Ferrata), or *lymphoidocyte* (Pappenheim) for this cell."

"The dualists believe that the granuloblast (myeloblast), monoblast, lymphoblast, and plasmablast, as pictured, are actually one and the same cell which is capable of giving rise to any of the leukocytes but that the megaloblast is a distinct cell type, capable of giving rise to cells of the erythrocyte series only. The leading exponents of this school are Ehrlich, Naegeli, Schridde, Piney and Helly."

"The trialists, among whom are Schilling and Rosenthal, differ from the dualists only in believing that the monocyte series is derived from a reticuloendothelial cell of the spleen (not the monoblast that is here depicted). They agree with the dualists that the other leukocytes are derived from a single stem cell, usually called a *myeloblast,* and the erythrocytes from a third stem cell, usually called a *megaloblast.*"

"The extreme polyphyletists, represented by Sabin and her students, believe each of the series is derived from a separate stem cell somewhat as here pictured."

Osgood has apparently solved all the problems of hematology, for "The author believes that both the monophyletists and polyphyletists are right. It is absolutely certain that all of the cells are originally derived from a fertilized ovum and probably much later in embryonic life there is a single cell capable of giving rise to either a granuloblast (myeloblast), monoblast, lymphoblast, plasmablast, or karyoblast (megaloblast). This single cell probably is either an embryonic reticulum cell or an embryonic endothelial cell. It is the belief of the author that once the stem cells have been formed, they lose the ability to differentiate into any other cell than those in the line of development in the frontispiece."

No one will disagree with him that all of the cells are originally derived from a fertilized ovum.

With regard to the monocytes, Osgood states on page 32: "They (monocytes) are found also in the spleen and in the tissues where they are commonly called *histiocytes* or *resting wandering cells.* Sabin and others believe that this tissue cell differs from the monocyte usually found in the blood and call the tissue cell a *clasmatocyte.* In the author's opinion the differences in appearance are due to differences in function and activity and not to any real difference in essential function." That he is identifying the monocytes of the blood with the clasmatocytes or fixed phagocytes of the spleen and other hemopoietic organs is obvious since on page 156 one finds this statement: "It seems possible that Hodgkin's disease will prove to be a malignant tumor of the fixed phagocytic cells of the monocyte series . . ."

There are several unusual hints in distinguishing various cell types: On page 32, under monocyte *43,* he writes, "By holding the illustration far enough from the eye so that the individual granules are no longer visible, the dirty gray appearance of the cytoplasm of these cells when seen with a poor stain or through dirty or poor lenses is evident." On page 144, under "Interpretation of the Peroxidase Stain," he gives the following suggestion as to how to distinguish between prolymphocytes and progranulocytes: "If the sum of all neutrophils, eosinophils, basophils and monocytes (Wright's stain) is significantly less than the percentage of peroxidase positive cells, progranulocytes A (promyelocytes II) must be present in sufficient numbers to account for the difference." Numerous similar examples of ways of differentiating the earliest forms of the granulocytes from one another, based not on morphology but on observation of the accompanying more mature cells, vitiate many of the aims of, and claims for, the atlas.

Some of the observations presented for the less common diseases of the blood are inaccurate. For instance, on page 100 it is stated that Trypanosoma gambiense is very abundant in the blood of a patient with African sleeping sickness and that it can hardly be mistaken for anything else. But on page 203 it is stated that one sometimes finds it necessary to concentrate the blood in order to find the parasite. Despite the fact that one has been told that T. gambiense can hardly be mistaken

for anything else, on page 203 one is told that Trypanosoma cruzi can be differentiated from T. gambiense "by the difference in geographical distribution and the clinical symptoms, although there are also slight morphological differences in the appearance of the organisms."

The book was intended for the "clinician, student and technician" rather than for the hematologist. The atlas part alone might be of aid to the technician if it were not for its confusing terminology and its didactic aspect. For the clinician and the student it is misleading. They need to know the background if they are to understand the diseases from a pathologic and a physiologic point of view. And a text, such as this, which would satisfy the curiosity of a technician would not satisfy that of a modern physician.

The present reviewer agrees with the reviewer of the book whose review appeared in *The Journal of the American Medical Association* (**110**:392, 1938) to the effect that "few hematologists or those who have had experience with hematologic work will share the senior author's rather optimistic view that this book, a microscope and a patient will solve a large part of the vicissitudes of hematology."

The Patient and the Weather. By William F. Petersen, M.D., with the assistance of Margaret Milliken, S.M. Volume IV, Part 3. Organic Disease: Surgical Problems. Price $10. Pp. xxxvi and 651, with 482 illustrations. Ann Arbor, Mich: Edwards Brothers, Inc., 1938.

Part 3 of volume 4 of Petersen's monographs entitled "The Patient and the Weather" is an imposing tome that is directed primarily to the surgeon. It deals chiefly with those acute conditions, usually considered infectious in origin, that generally fall within the province of the surgeon. Petersen attempts to combat in the mind of the surgeon the dominance of the concept of infection as an etiologic factor. He states his point of view in this respect quite frankly (p. vi): "The 'acute' surgical conditions are not primarily due to bacterial infection. They are primarily due to vasomotor and neuromuscular dysfunction. Bacteria play a secondary role and are adventitious."

The surgeon is a busy man "who thinks of disease as a 'thing in itself,' an isolated expression of a localized phenomenon, something to be removed in toto, and its local pathogenesis as separate from the organism" (p. x). He has therefore been spared the necessity of reading the previously published 3,267 pages to gain an insight into the way man's destiny is controlled by his meteorologic environment through his autonomic mechanisms. The first chapter, 130 pages subdivided into 4 sections, restates for the surgeon the author's concept of reactions to environment. In the determination of infection and inflammation the autonomic reactions play a fundamental part.

In succeeding chapters such acute conditions as gastric ulcer, cholecystitis, appendicitis, pancreatitis, gastrointestinal lesions and ectopic pregnancy are discussed not from the standpoint of surgical operative technic but as episodes in which meteorologic changes are the immediate provocative factors. The discussion of acute appendicitis fails to distinguish between acute obstructive gangrenous appendicitis and what may be termed acute infectious appendicitis. One may readily agree with Petersen that the latter form is initiated by muscle spasm and vascular reaction, on which bacterial penetration and localization depend, but the two forms of acute appendicitis are clinically, pathologically and prognostically distinct. It would be more life saving for surgeons to recognize these differences than to know that an autonomic reaction may be at the bottom of each type.

After an operation the patient is even more susceptible to the weather than before. Postoperative complications and vascular accidents, such as thrombosis and embolism, are discussed as states precipitated by the weather. The subject matters of certain special surgical fields, e. g., abscess of the brain, orthopedic diseases and ophthalmologic episodes such as glaucoma and retinal hemorrhage, come in for their share of attention.

Then follows a chapter devoted to the endemiology of the surgical diseases. Incidence, morbidity and mortality in this country and in Europe are correlated with seasonal cycles and with the longer sun spot cycles. Perhaps the future will have to take into account even longer interplanetary system cycles. The final chapter is a brief recapitulation of the matter covered in this and the preceding volumes and parts of volumes. It forms a sort of reader's guide which he might do well to read before he lays out the seven volumes for an evening's enjoyment.

In this part 3 of volume 4 the surgeon is told many things, some of which he may consider theoretic, but he is not dismissed without some direct advice. "In America the late winter and early spring periods offer an excessive hazard and one that cannot be obviated by the mere administration of a little alkali before the operation. In America more than elsewhere surgeons should take their vacations in the late winter and spring. The mortality rates would be lessened" (p. 441). Further admonition reads "certainly no surgeon who is a true physician would subject a woman to an operation premenstrually when delay to the postmenstrual phase could be arranged" (p. 441).

In this surgical monograph Petersen has dealt with the reactions of the patient to the meteorologic environment. But the surgeon too is a "cosmic resonator," like his patient, and sometimes is even more resonant. He too pendulates from ARS to COD (and sometimes even to cash in advance). A polar infall might therefore make a handy alibi for such occasional errors in judgment as surgeons sometimes make.

Usage has finally made this reviewer succumb, after a long life of inward protest which is not yet stilled, to the use of "data" in the singular. But with a Martin Lutherish "here I take my stand until I die" he draws the line at "a vasomotor phenomena" (p. vi) and "the bacteria and its viability" (p. 536). "A urethral calculus was observed in the lower third of the right ureter" (p. 485) would appear to be a process akin to retrograde embolism. One winces a bit internally at the use of such surgical solecisms as "the acute appendix," "the acute abdomen," "the patient was operated," but since the author is talking to surgeons perhaps he considers it best to speak their language. Of the case records, many have been reproduced from the literature. The reduction in reproduction has made the type so small as to make reading a strenuous job.

The Biology of Arteriosclerosis. By M. C. Winternitz, R. M. Thomas and P. M. LeCompte, Department of Pathology, Yale University School of Medicine. Pages 122, with 116 illustrations, including 58 in color. Price, $4. Baltimore: Charles C. Thomas, 1938.

The text of this monograph is concise and clear, and the illustrations are excellent. The pathology of arteriosclerosis is presented from a new standpoint, which is based on the following technic: The intrinsic blood supply of the arterial wall is demonstrated by an injection of Higgins' engrossing ink filtered through coarse filter paper and diluted eight times with distilled water. Both the ink-prepared and the unprepared arteries are cleared with glycerin or with Spalteholz' fluid and examined under low magnification. Microscopic sections are also prepared. By this technic it is shown that the mural arterial vessels arise from three separate sources, namely, the adventitia, the region of the orifices of branches and directly from the lumen of the vessel. Relatively few vessels are commonly found in the inner media and intima of normal arteries of young persons, but an abundant vascular supply is demonstrable in diseased arteries. The vascular network is abundant about arteriosclerotic intimal lesions. The authors attach great importance to mural hemorrhages, which occur with great frequency in the vascular plexuses. The hemorrhages occur especially about the origin of branches, where the vascular network is abundant, and it is suggested that this relationship explains the early occurrence of atheromatous lesions at these sites. The authors are inclined to believe that mural hemorrhages are the initial lesions in arteriosclerosis.

They explain many of the histologic features, such as macrophages, lipoid phagocytes and tissue proliferation as a reaction to the hemorrhage, and they suggest that arteriosclerosis may be of inflammatory origin.

The authors have presented an excellent study of the vascular supply of the arterial wall and its alterations in disease, but their theory that intramural hemorrhage is the basic lesion is not sufficiently well grounded. It appears equally possible that the increased vascularity is a secondary response to the atheromatous lesion rather than its initiating factor.

The monograph will be of great interest to all who are interested in the fundamental nature of arteriosclerosis.

De l'influence de divers cations sur le croît microbien. Lucien Neipp, D.Sc. Price, 90 francs. Pp. 430, with 5 tables and 202 graphs. Paris: Masson & Cie, 1937.

This research monograph deals with the influence of various metallic salts on the growth of bacteria from the standpoint of the Arndt-Schulz law and the oligodynamic effect of metals. The author criticizes previous experiments on the ground of inaccuracy in measuring growth or of insufficient consideration of phases of growth. Preliminary to a discussion of his own experiments, he presents an extensive critical review of methods for measuring bacterial growth, of the phenomena expressed in the growth curves of bacteria and of the influence of cations on the growth of bacteria. Accompanied by an extensive bibliography (over 500 items), this portion of the book is a valuable reference work for these fields.

Neipp investigated extensively and carefully the effect of the nitrates of lanthanum, cerium, lead, mercury and silver on the rate of growth of Pseudomonas aeruginosa in a dialyzed peptone medium. All except silver nitrate exhibited three effects: inhibition of growth at higher concentrations, no effect at intermediate concentrations and stimulation of growth at minimal concentrations. Silver nitrate showed no stimulating action at any concentration. Toxic doses extended the lag phase, shortened the growth phase and accelerated the death phase. With stimulating concentrations, there was no proportionality between the degree of stimulation and the concentration of the cations; neither was there any relationship between the degree of stimulation and the valence of the metal.

The author tried to determine whether the effect of the cations might be due to a modification of the electric charges on the bacterial cells. This portion of the book is again preceded by an extensive review of cataphoretic experiments with bacteria. He found that the rate of migration of the bacteria in an electric field was not changed by the concentration of the cells in the suspension, by the growth phase of the culture from which they were taken or by the addition of varying concentrations of lanthanum nitrate.

The experiments reported are more extensive, more precise and more carefully controlled than many of those which have been previously reported in this rather controversial field. It appears that the stimulating effect of minute concentrations of certain toxic substances may be considered to be well established but that this phenomenon is not sufficiently general to be designated a law.

Textbook of Experimental Surgery. By J. Markowitz, M.B., Ph.D., M.S. Cloth. Pp. 527, with 330 illustrations. Price, $7.00. Baltimore: William Wood & Company, 1937.

This is an interesting account of more than a hundred experimental procedures of a surgical nature. The author discusses in detail the care of animals, the necessary surgical apparatus and the technics of most of the commoner laboratory operations. He has written clearly and interestingly, with a liberal sprinkling of humor and philosophy. Throughout there is excellent correlation of the fundamental physiologic and pathologic implications involved in the surgical experiments. Especially strong are the sections on the abdominal viscera. The book is well illustrated.

This century has seen the origin and development of an important school of experimental medicine in which chronic procedures of the Bernardian type of physiology have been carried out under asepsis. The author of this book is a research associate in the department of physiology at the University of Toronto. Most of the experimental operations described have been devised by physiologists and pathologists. It is perhaps pertinent to inquire why professional surgeons have not taken a greater part in this movement. The reasons are simple. Surgery has in great part been dominated by clinicians, who have not had the necessary time for contemplation and execution of planned experiments. Moreover, there has been too little contact between the experimentalists and the clinical surgeons. In isolated instances in which association between these groups has been intimate, contributions of value to both have resulted. In epitome, the surgeon has suffered from too little leisure and from lack of stimulus, a mutually antagonistic combination. This book should provide a stimulus to the clinical surgeon and further the advance of academic surgery.

It is the fashion for book reviewers to point out defects. No serious blemishes are apparent in this volume. A few typographic errors are met, and in several cases the information is already out of date. This book should be in the hands of all investigators using surgical methods.

A Textbook of Histology. The Functional Significance of Cells and Intercellular Substances. E. V. Cowdry, Professor of Cytology in the School of Medicine, Washington University, St. Louis, Mo. Second edition, thoroughly revised. Cloth. Pp. 600, with 323 illustrations. Price $7. Philadelphia: Lea & Febiger, 1938.

In the preface to the second edition Cowdry states that the book has been criticized because it did not have the customary introduction, because it was written for students with less preliminary training than most of those who enter medical schools and because it was not illustrated adequately and was difficult to use in the laboratory. In the new edition an introduction has been added which, in part historical, is intended to guide students in their further work. But the level of presentation has not been lowered because obviously adequate preliminary training should be required. Many new illustrations have been introduced to show variations in structure dependent on degrees of physiologic activity and on aging. Finally, an attempt has been made to bridge the gap between gross and microscopic anatomy in order to make the book a better guide in the laboratory. In other words, an earnest effort has been made to improve the book in the light of the constructive criticisms that have been offered.

A Textbook of Bacteriology. Edwin O. Jordan, Ph.D., Late Andrew McLeish Distinguished Service Professor of Bacteriology in the University of Chicago. Revised by William Burrows, Ph.D., Assistant Professor of Bacteriology in the University of Chicago. Twelfth edition. Cloth. Price $6. Pp. 808, with 197 illustrations. Philadelphia and London: W. B. Saunders Company, 1938.

This book has been subjected to a thorough revision by William Burrows. Some sections have been rewritten, notably those on oxygen supply and bacterial respiration, on filtration, on infantile paralysis and on virus diseases and "virus proteins." Epidemic influenza has been moved to the chapter on virus diseases. The section on yellow fever has been amplified to include the newly discovered jungle fever and other advances in the knowledge of the disease. The chapter on pathogenic protozoa has been brought up to date by William H. Taliaferro. The size has not been increased, however, and the book continues to be a model "in the concise clearness, the judicial restraint and the remarkable accuracy of the presentation."

Books Received

A TEXTBOOK OF HISTOLOGY. Functional Significance of Cells and Intercellular Substances. By E. V. Cowdry, Professor of Cytology in the School of Medicine, Washington University, St. Louis, Mo. Second edition, thoroughly revised. Cloth. Pp. 600, with 323 illustrations. Price $7. Philadelphia: Lea & Febiger, 1938.

THE PATHOLOGY OF DIABETES MELLITUS. By Shields Warren, M.D. With a foreword by Elliott P. Joslin, M.D. Second edition. Cloth. Pp. 246, with 89 illustrations. Price $4.75. Philadelphia: Lea & Febiger, 1938.

DIE FETALE UND POSTFETALE TUBA EUSTACHII. ANATOMISCH-HISTOLOGISCHE UNTERSUCHUNGEN. By Adolf Schwarzbart. Paper. Pp. 160, with 80 illustrations. Cracovie, Poland: Imprimerie de l'Universite, 1938.

PATHOLOGY AND MICROBIOLOGY. Chinese Medical Journal Supplement II. Issued by the Chinese Society of Pathology and Microbiology on the occasion of the visit to China of Dr. Hans Zinsser. Paper. Pp. 600, with illustrations. Price $2.50. Peking, China: Chinese Medical Journal, 1938.

SURGICAL PATHOLOGY. By William Boyd, M.D., LL.D., M.R.C.P.Ed., F.R.C.P. London, Dipl. Psych., F.R.C.S., Professor of Pathology, University of Toronto. Fourth edition, revised. Cloth. Pp. 886, with 491 illustrations. Price $10. Philadelphia and London: W. B. Saunders Company, 1938.

LABORATORY MANUAL OF HEMATOLOGIC TECHNIC. By Regena Cook Beck, M.A., M.D., Formerly Instructor in Pathology and Bacteriology at George Washington University Medical School; Head of the Department of Bacteriology, William and Mary College Extension; Pathologist to the Stuart Circle Hospital and Director of the Stuart Circle Hospital School of Medical Technology, Richmond, Va. With a foreword by Frank W. Konzèlmann, M.D., Professor of Clinical Pathology, Temple University, Philadelphia. Cloth. Pp. 389, with 79 illustrations. Price $4.00. Philadelphia and London: W. B. Saunders Company, 1938.

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